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The effects of heavy metal pollution on woodland leaf litter faunal communities

THE EFFECTS OF HEAVY METAL POLLUTION ON WOODLAND LEAF LITTER FAUNAL COMMUNITIES.

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A thesis submitted to the University of Bristol in accordance with the requirements for Doctor of Philosophy in the Faculty of Science.

The research reported within this dissertation is entirely my own work except where otherwise stated,

Helen J. Read.

I certify that the above statement is true.

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ABSTRACT.

Six woodlands with differing degrees of heavy metal pollution have been studied. The site descriptions include measurement of metal concentrations (cadmium, copper, lead and zinc) in various soil layers and deposition rates.

The worms and microarthropods show variation in numbers between the different sites but not related to the pollution. Two species of macroarthropod, one woodlouse and one millipede, showed elevated levels of metals when originating from the polluted site; little variation over the year was detected. In laboratory experiments several species of millipede were fed clean or contaminated leaves. Juveniles accumulated metals to a greater extent than adults. In all species studied juveniles showed reduced survival and reduced growth rates on contaminated leaves.

Several species of small mammal were caught and metal concentrations determined. Shrews contained higher concentrations than other species especially of cadmium. Adult *S. araneus* had higher concentrations than adult *S. minutus*. This was attributed to different overwintering diets.

The macroarthropods were sampled in a large scale program of pitfall trapping. Diversities (H´) varied for each group of animals but overall there was a tendency for diversity to decrease closer to the pollution source. Numbers of individuals and species did not show such a clear trend.

Results were analysed using canonical correspondance analysis (CCA). The effect of the heavy metal pollution was found to have a large influence on the communities. Species could be identified which associate with or disassociate from high levels of metals. The most polluted site had an identifiably different invertebrate fauna. It is concluded that a small number of species are able to survive at the polluted site. A much larger number are potentially able to live in the cleaner sites. Their distribution is determined by other factors. An attempt is made to determine why some species are able to survive in polluted environments. They are not necessarily those which are expected. Lack of potential competitors may have an influence.

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"The following principals are revolutionary because they depend on common sense not science.
People like to identify things.
It is possible to identify anything.
Identification is made easier not by offering more choice of models but less choice.
No part of nature should be divided into more than ten species."

Miles Kington (1983). Nature made *ridiculously simple* or how to identify absolutely anything. Penguin books, Middx., England.

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Chapter 1.

INTRODUCTION TO THE THESIS.

1.1. General.

The forest floor and its associated soil is a very important and complex part of the woodland ecosystem. As well as harbouring the root systems of the vegetation, it receives large quantities of organic debris in the form of leaves, fruit, flowers and stems of the plants, particularly the trees. Although the underlying soil is usually derived from the bed rock of the area, the large input of organic matter, often on a seasonal basis, exerts a large influence and maintains a further important component of the ecosystem comprising invertebrates and decomposer organisms.

Within the soil and the leaf litter are large communities of animals, to which many studies have been devoted (e.g. Burgess & Raw 1967, Kevan 1955, Doeksen & van der Drift 1963, Phillipson 1971, Murphy 1962, Kuhnelt 1976, Wallwork 1970, 1976). In size these organisms range from protozoa to burrowing mammals, encompassing many taxonomic groups. The microfauna (Fenton 1947), up to 200µm, consists principally of the protozoa. The mesofauna or meiofauna (Murphy 1955), ranges from 200µm to 1cm and includes the mites, Collembola, nematodes, enchytraeids and small spiders. The first four of these groups are active in the decomposition process, although some species may feed on living vegetation or are predatory. The spiders are principally predators. The macrofauna (Fenton 1947), in size between 1 and 2cm, is a very diverse grouping. Included are the earthworms, diplopods, isopods and molluscs, all principally decomposers, and the beetles (predators or herbivores) and other arachnids (predators or scavengers). The megafauna (Van der Drift 1951) are larger than 2cm and consist of the large earthworms and the small mammals. In the present study, emphasis has been placed on the decomposers and selected carnivores, principally spiders, Carabid beetles and shrews. Extensive study of the microfauna, although undoubtedly of importance in the soil ecosystem, is beyond the scope of this investigation.

The effect that different types of pollution are having on the environment is a topic of increasing public interest. The industrialisation of the western world has inevitably produced waste products which are difficult to dispose of and which, frequently are released, albeit in low quantities, into water or into the air. Discharge into the air leads to consequences which are only slowly being realised; the effect on the upper atmosphere (World Meteorological organisation 1985) and the acidification of subsequent rainfall (Hutchinson & Havas 1980) are two of the most notable problems. Particulate matter released must eventually fall to the ground and will be incorporated into the soil. Potentially toxic compounds and substances are frequently part of this matter and include heavy metals.

The metals with which this thesis is concerned (cadmium, copper, lead and zinc) are concentrated in the environment by a variety of means (Freedman & Hutchinson 1981). Discharge from industrial areas is of course one, as is exhaust fumes from vehicles. Application of sewage sludge to the land adds high concentrations of some metals to the soil and mine spoil tips are also sites where concentration of metals has taken place (Purves 1977). These examples give an indication of the situations available for study and all have been observed in some way from a biological viewpoint.

The effect of heavy metals on the environment has been given in more detail in books edited by Nriagu (1978a,b,1979, 1980a,b,c,d,e,f) and Friberg *et al*. (1971) and the effects on plants presented by Lepp (1981a,b). With regard to animals there are extensive small surveys although not as yet a comprehensive treatise. Useful reviews are provided on the effect of pollution on communities by Sheehan (1984a,b) although pollution of aquatic environments occupies much space. Biological monitoring of heavy metals in air and terrestrial environments has been dealt with by Martin & Coughtrey (1982). The emphasis so far on animals has mostly been the chemical analysis of the amount of metal in the organism or parts thereof. Links in food chains have in the main been solely between one species and its food. The effect of heavy metals on groups of animals or the functioning of communities as a whole has been far less extensively reported.

The presence of a major primary lead-zinc smelting works at Avonmouth near Bristol has stimulated research in the local environment since the early 1970's (for a review of previous work see chapter 2). In particular work has centred on a pair of woodlands, which receive heavy fallout of the metals lead, zinc, cadmium and copper. Attention was drawn to the decomposer community in these woods because of the large depth of leaf litter (Coughtrey *et al.* 1979). The implication of the increased leaf litter to the decomposer fauna is considerable. The aims of the present study were to sample the decomposer community in one of these woods and to compare it to other similar woodlands in the area, with differing amounts of metal pollution in order that the effect on this habitat could be quantified. In addition to sampling the decomposers, some of the predators, both invertebrates and vertebrates, were sampled in order to examine the impact of the pollution on a higher trophic level in the same system.

1.2. The structure of the thesis.

The thesis is organised such that chapter 2 provides a summary of the literature of heavy metal pollution in soil communities. Previous work on the effects of pollution from Avonmouth is also summarised. Chapter 3 presents the methods used. Principally atomic absorption spectrophotometry for determining metal concentrations and pitfall trapping for obtaining animals. Chapter 4 is a detailed description of the sites used for sampling. This includes details about the vegetation and attempts to classify the sites using available methods. Metal concentrations in the soil layers are given and the results of moss bags, used to measure the rate of deposition of the pollutants. In chapter 5 the effects of heavy metal pollution on decomposers is considered. In particular with reference to the worms, microarthropods, millipedes and woodlice. The small mammals captured are discussed in chapter 6, both in view of the numbers caught and the metal concentrations within them. Chapter 7 takes a wider view of invertebrate communities and explores various methods of looking at them. One of those used, canonical correspondence analysis, is a relatively novel method which seems particularly appropriate to the current situation.

Each chapter or section thereof incudes a discussion and a summary, however chapter 8 attempts to draw the findings together and presents a general overview

The appendices contain raw data and minor sections of relevance to the thesis but too voluminous to include in the main text. A large body of data, that of the pitfall captures is not presented. This may be obtained from the author c/o Dr. M.H. Martin on request.

Three papers have been published (or are in press) relating to the work reported in this thesis. All three are given in appendices. An additional paper in manuscript form only is also presented.

1.3. Notes on the terms used.

The term ´heavy metal´ is perhaps an over used term which in most literature is taken to mean metals with specific gravities of greater than 5. Nieboer & Richardson (1980) have proposed another classification which is more biologically and chemically based. Of the three groups proposed, zinc and cadmium fall in the borderline (or intermediate) category whilst lead and copper are either borderline or class B (nitrogen/sulphur seeking), according to the valence of the ion involved. As this is rather clumsy to express and has not been widely adopted by authors, the term ´heavy metal´ has been maintained in the thesis.

Due to the large number of species names referred to in the thesis, authorities have not been given in the text, but Table 1.1 indicates which literature each group follows.

In the thesis a variety of statistical methods have been used, some of which are discussed more fully in appendix 1. Two principal statistical packages were used, Minitab (Ryan *et al*. 1985) run on an Opus P.C.II and on Bristol University's main frame 'Multics', and Canoco (Ter Braak 1987a) on the Opus. Graphs were produced using 'Tel-a-graf' on 'Multics'. Where appropriate means ± S.E. are quoted. Metal concentrations are given in $\mu g/g^{-1}$ dry weight unless otherwise stated.

Table 1.1

SPECIES NAMES USED IN THE THESIS FOLLOW THE APPROPRIATE TEXTS GIVEN BELOW (EXCEPT FOR THOSE SPECIES INDICATED AT THE END).

Group	Reference
Vascular plants	Clapham, Tutin & Moore (1987)
Bryophytes	Smith (1978)
Worms	Sims & Gerard (1985)
Molluscs	Kerney & Cameron (1979)
Woodlice	Harding & Sutton (1985)
Millipedes	Blower (1985)
Centipedes	Eason (1964)*
Harvestmen	Savory & Sankey (1974)*
Spiders	Roberts (1987a)
Beetles	Kloet & Hincks (1977)
Amphibians	Arnold & Burton (1978)
Mammals	Corbet & Southern (1977)

*Exceptions:-

Centipedes

Lithobius microps (Meinert) = *Lithobius duboscqui*

Lithobius borealis (Meinert) = *Lithobius lapidicola*

Harvestmen

Rilaena triangularis (Herbst) = *Platybunus triangularis*

Lophopilio palpinalis (Herbst) = *Oligolophus palpinalis*

Paroligolophus agrestis (Meade) = *Oligolophus agrestis*

Chapter 2.

INTRODUCTION TO THE RELEVANT LITERATURE.

2.1. Decomposition.

2.1a. General.

Within a woodland ecosystem, organic matter in the form of leaves, stems, twigs, branches, bud scales, fruit and flowers, together with animal products reaches the surface of the soil, whilst roots and soil animals contribute to the organic matter within the soil. The progressive breakdown of the organic matter including the processes of comminution and decomposition releases plant mineral nutrients into the soil and enables their recycling. The breakdown and redistribution of organic litter and root material is the most important role of soil organisms and a wide diversity of animal species are involved in this process.

The organic litter layer of soils is usually approximately 1.5 times the annual leaf fall, which in a stable forest is surprisingly constant (Burgess 1967). The components of the litter will vary according to the region and the type of wood. On average 60-70% is leaf material, 12-15% is branches, and fruit and bark compose 1-15% each (Bray & Gorham 1964). Litter from angiosperm forests in temperate regions commonly consists of 21% non leaf litter (Bray & Gorham 1964). Whilst leaf litter may decompose in 2 to 4 years, wood takes much longer (Healey & Swift 1971). The decomposition of wood involves a different community of animals and plants and much of the process may take place in the canopy before the wood reaches the forest floor (Swift et al. 1976). Sampling wood decomposers requires different techniques to those used in the present study and most of the attention in the rest of the thesis is devoted to leaf litter decomposers. In the decomposition of leaf litter two main processed take place; comminution, which is the progressive breakdown of organic items into smaller fragments and humification, the binding together of organic and mineral particles.

A large proportion of the ground community is involved in the process of decomposition. Reichle et al. (1975) have estimated that 71% of the CO_2 efflux from a forest floor (of a deciduous wood) was due to the decomposer biota. A large number of groups are involved in some way and Begon et al. (1986) have commented that,

"Decomposer communities are, in their composition and activities, as diverse as or more diverse than any of the communities more commonly studied by ecologists."

For a deciduous wood a brief synopsis of the process of decomposition is as follows:-

Colonisation of fungal growth may start on a leaf before it falls from the tree. It is then attacked by a succession of fungal species, the hyphae of which are active mostly on the internal tissues of the leaves (Gray & Williams 1971). During the first winter after leaf fall, fungi and protozoa may attack the leaves but in the early spring the rate of decomposition speeds up with the increasing activity of the arthropods (Kuhnelt 1976). Millipedes and woodlice consume vast quantities of litter. The action of eating macerates the leaf cell walls and all the indigestible contents are excreted as faecal pellets, together with the metabolic waste products (Russell 1969). The faeces produced by the macroarthropods are in turn consumed by the secondary decomposers principally the microarthropods. The action of the earthworms, and also some of the macroarthropods, mixes the soil layers. Earthworms in addition feed on the leaf litter and excrete waste products in the form of casts. The casts consist of aggregates of soil and organic matter (Guild 1955), to which plant nutrients associate (Kuhnelt 1976) and in this form are available to plants.

This may sound very clear cut and straight forward, however the relative roles of the various organisms is, in many cases, in dispute. One way to help clarify this is to use some method of excluding sections of the fauna and observe any differences from when all are present. Two main methods have been used to achieve this; putting litter in bags of different mesh size, so allowing different sized organisms in, and adding naphthalene which excludes the invertebrates but not the micro-organisms. By these methods it has been shown that exclusion of the invertebrates slows down the rate of decomposition (Kurcheva 1960). Witkamp & Crossley (1966) showed that reducing arthropod numbers by 82% increased the bacteria population to 6 fold the original but there was a reduction in the rate of weight loss of the litter of 36%.

Earthworms are frequently considered to play a major role in decomposition (e.g. Hayes 1983). They effect three main types of action on the soil and litter (Guild 1955); they ingest organic matter and help in the breakdown to smaller pieces. The ingested organic and inorganic matter is mixed and then egested as casts containing clay-humus complexes ie. they are agents of humification. Finally they act as mixers and aerators of the soil. However Kuhnelt (1976) comments that it is easy to give worms ´pride of place´ and to underestimate the importance of other animal groups.

Some of the macroarthropods may also have similar roles to the earthworms. Both diplopods and isopods act as mechanical processors of leaf litter. As well as macerating and breaking down cell walls (Russell 1969) they increase the surface area available for microbial attack by up to 4.4 times the original surface area (Hassall & Sutton 1978). Whilst it is generally considered that cellulases are not present in the gut of many of the larger invertebrates (Wallwork 1970), Hassall & Jennings (1975) have shown that microbes that are taken in with the food can exist in the guts of isopods and break down cellulose. The guts of animals provide good environments for microbial growth. Anderson & Bignall (1980) have shown that the passage through the gut of *Glomeris marginata* (Diplopoda) enhances the growth and viability of bacteria. Wieser (1966) considered that isopods might be of importance as secondary decomposers, feeding on faeces, due to their habit of coprophagy.

The possibility that diplopods may form excrement aggregates like worms has been suggested (Blower 1955, Bocock 1963, Marcuzzi 1970, Kuhnelt 1976) but is disputed by Striganova (1971) who considered that only earthworms and some Diptera larva were capable of it, and van der Drift (1951) who reported *G. marginata* to feed on the micro-organisms on the leaves and not to transform the leaves in any way. There is no doubt however that these animals do have a large effect on the litter. Gilyarov (1970) reported that diplopods and isopods convert 10-30% of the annual litter fall to faeces and that 80-95% of the ingested food is egested.

The microarthropods (mites and collembola) feed on a variety of foods. Collembola are principally mechanical destroyers (Striganova 1971) mostly feeding on fungi, bacteria and algae (Gilyarov 1970). The mites, a very diverse group, include predatory and herbivorous species, however the order Cryptostigmata includes animals mostly concerned with litter breakdown (Evans et *al*. 1985). Species in this group feed on micro-organisms or decaying plant material or both

(Gilyarov 1970). Anderson (1975) records evidence of trophic separation in the group according to body size and reports a succession of species diversity in litter from fresh leaves to humus. Whilst mites and Collembola are considered groups of active litter destroyers and again to increase the surface area of litter (Gilyarov 1970) they do not alter the litter chemically (Striganova 1971). Swift *et al*. (1979) considers their sum contribution to litter breakdown to be insignificant but Madge (1969) has shown that they can be very important.

Nematodes and enchytraeids can also be numerous in the soil, helping with soil aeration and feeding on plant material. Enchytraeids may be of importance in the breakdown process as they have been shown to excrete very little cellulose in the faeces, so they may complete the decomposition of this substance (Striganova 1971).

The biomass of protozoa in soil in deciduous woods has been estimated at 100kg/ha. By feeding on bacteria they have a large indirect role in the community (Gilyarov 1970). This is also true of the larger predators of soil organisms which may have a regulatory influence on other animals and so effect decomposition (Swift *et al*. 1979).

The action of the invertebrates as a whole is to reduce the particles of litter to a progressively smaller size i.e. comminution, gradually increasing the surface area for microbial attack. Nicholson _et al_. (1966) found that the rate of decomposition of the faecal pellets of _G. marginata_ was the same as that of hazel litter, however it is probable that many of these pellets will pass through the guts of other invertebrates. The arthropods may not always be feeding directly on the dead material but grazing on the micro-organisms growing on the debris or faeces. The substrate is ingested but is a less important food source (see Begon _et al_. 1986 for more details).

The activity of all the organisms in the soil contributes to the process of decomposition in some way (cf. the predators). It has been suggested that organisms that are very active but have short lives may contribute more to the community than those which are larger, live longer and form larger proportion of the biomass (Begon _et al_. 1986). The interaction of the larger detritivores and the microbes is fundamental and both are obviously necessary for speedy decomposition.

There are various factors which alter the rate at which decomposition takes place and, if it is very slow, the increased rate of accumulation of organic matter. Whilst the failure of decomposition is not generally considered beneficial, Begon _et al_. (1986) have pointed out that it is this failure which has produced peat, oil and coal.

The quality of the resource (Swift et al. 1979) can affect the rate of decomposition. It has been shown many times that decomposers have preferences for particular species of leaves and different ages or treatments of them. The factors governing these preferences are more difficult to ascertain. Nitrogen content has been considered important and the carbon to nitrogen ratio (Marcuzzi 1970, Wallwork 1976), toughness, phenol content and surface texture of leaves have all been considered (Swift et al. 1979). Calcium content was also related to palatability in millipedes (Lyford 1943). A second group of influences on decomposition are the physico-chemical ones (Swift et al. 1979); these include aeration, leaching, temperature, moisture (Bond et al. 1976, Howard & Howard 1980) and pH (Baath et al. 1980). Heavy metal pollution undoubtedly has an effect too (see below).

The relative presence of different groups of organisms affects the rate of decomposition. In general there is a correlation between the numbers and composition of the soil fauna and the rate of decomposition (Gilyarov 1970). If animal activity is low there is little humification (Kubiena 1955). In forest soils that are well mixed there is little superficial organic matter (mull) and there is usually a high proportion of large invertebrates such as worms and millipedes. In contrast in soils with large proportions of organic matter showing distinct layers above the mineral soil (mor), there may be a large number of arthropods, of which by far the majority will be micro-arthropods (Murphy 1955, Burgess 1967, Wallwork 1976). These two different forest soils, mull and mor, are generally found under different types of tree (deciduous and coniferous respectively) and represent two extremes. The formation of the two types was described in detail by Handley (1954). Between the extremes other types can be recognised including moder. In moder soils, the leaf litter is broken down into smaller fragments but mixing and humification by the larger arthropods is lacking (Kubiena 1955). In summary Swift *et al*. (1979) said,

"The climate sets the upper and lower limits to the potential decay rate, the ´fine control´ at the local level is determined by resource quality and factors of the edaphic complex."

2.1b *Effect of heavy metals*.

It has been noted before, in many situations where high concentrations of heavy metals occur that there is an accumulation of leaf litter. Coughtrey *et al*. (1979) found that accumulation was not at the initial stages of breakdown and the bulk consisted of particles 1-8mm in size. Watson (1975) also reported that accumulation was of older fragments. As decomposition proceeds, metal concentrations increase mostly due to the decreasing weight (Nilsson 1972, Inman & Parker 1978) although some nutrients may be extracted (Zielinski 1984). A slowed rate of decomposition is not generally beneficial as nutrients are bound into the leaf litter (Tyler 1972).

The rate of decomposition can be measured in a variety of ways. The fact that polluted litter accumulates has been established using weighed litter bags. Zielinski (1984) reported that in polluted areas 43.4% of leaves in bags had not decomposed after two years and Inman & Parker (1978) found that litter at an unpolluted site lost two times the weight of that at a similar urban polluted site during 11 months. Indication of the rate of decomposition can also be obtained by measuring carbon dioxide evolution from the respiring organisms undergoing the decomposition process. Using this method Chaney *et al*. (1978b) showed reduced activity at polluted sites and created a similar effect by adding heavy metals to clean litter. Tyler (1975, 1976) also recorded reduced mineralization of nitrogen and phosphorus and reduced activity of ureases where copper and zinc concentrations were high in the top soil.

One interesting point noted several times is that low concentrations of metals (eg. Cd) can cause a stimulatory effect on the rate of decomposition (Bond *et al*. 1976).

In acidic soils the rate of decomposition can be slowed by lower concentrations of metals than in less acidic areas (Ruhling & Tyler 1973) but pH is not necessarily the cause of the slowed decomposition. In Gusum, Sweden, the region polluted by copper and zinc is less acidic than the control areas but has a deeper litter layer (Tyler 1976) i.e. more accumulation.

There is little dispute that heavy metal pollution slows the decomposition rate, resulting in an accumulation of leaf litter, but which part of the decomposer community is principally affected is more difficult to ascertain.

Jordan & Lechevalier (1975) have reported reduced numbers of total bacteria, actinomycetes and fungi close to a smelter and this was proposed as a cause of slowed decomposition by Tyler (1976) also. In contrast Killham & Wainwright (1981) found bacterial activity not to be inhibited by pollution. Microbes have been found to be tolerant to metals (Williams et al. 1977). Jordan & Lechevalier (1975) also recorded tolerance in the dominant species occurring near the smelter in their study but noted a difference in the species found in differentially polluted areas. Bond et al. (1976) also reported shifts in the populations in polluted soils but with no overall numerical changes (the latter was however, in laboratory microcosms rather than the field).

The relationship between numbers of arthropods and decomposition in metal polluted areas has also been investigated. Strojan (1978a) buried litter bags for up to a year, recording weight loss and arthropod numbers. A 19.1%

weight loss at a polluted site in comparison to 36.8% at a clean site was recorded and the density and diversity of arthropods was reduced in the polluted site, which contained only 18.1% of the total arthropod fauna of the clean site. The decline was mostly due to the numbers of mites. Killham & Wainwright (1981) also found a reduced arthropod fauna at a polluted site. In contrast, Freedman & Hutchinson (1980b) found the soil flora to be variable and not necessarily lower in polluted areas. At the woodlands at Avonmouth, no reduction in the microbial fauna has been recorded (Martin et al. 1978, 1980).

2.2 The situation at Avonmouth.

2.2a The source of pollution.

The area of Avonmouth is north west of Bristol, where the river Avon joins the Severn. It is well situated for import and export of bulky materials and has developed as an industrial area. There are several large chemical industries situated here, but the most important in terms of emissions of lead, zinc and cadmium into the atmosphere is the Commonwealth Smelting works.

Zinc smelting commenced on this site in 1928 (Coy 1984), although prior to this there were other industries producing effluent containing metals. In 1968 the smelting works was expanded and production was transfered to a new furnace. As well as zinc smelting, the expansion program included a cadmium plant, sulphuric and phosphoric acid plants and an effluent treatment plant (Little 1974). The new complex, including the largest furnace of its type and the only primary zinc and lead smelter in Britain (Coy 1984) suffered serious running problems which caused frequent breakdowns and stoppages. Attendant with these were spillages and subsequently, concern about lead poisonings culminated in the forming of a committee of enquiry, headed by Sir Brian Windeyer which then submitted a report to parliament (H.M.S.O. 1972). This report proposed recommendations for improvements affecting the health of workers. However it was considered that environmental problems were outside the scope of the committee. It was noted that the company (then Rio Tinto Zinc) had purchased the farm land adjacent to the works in order to control its use.

In 1984 the production capacity of the plant was 100,000 tons of zinc, 45,000 tons of lead, 400 tons of cadmium and 180,000 tons of sulphuric acid. The emissions from the stacks were $4.0kgh^{-1}$ of lead, $6.0kgh^{-1}$ of zinc and $0.4kgh^{-1}$ of cadmium. There are other sources of atmospheric pollution from the works, mainly dust from raw materials either in transit or when at the dump; these inputs into the atmosphere are not easily measured (Coy 1984).

Whilst the smelter is not the sole source of these metals in the area, it is the chief one. The construction of a refuse incinerator in the mid 1970's also increased the level of pollution. It is of notice that the area surrounding this industry has been subjected to contamination by various metals for over 50 years. Studies based at the University of Bristol and elsewhere in the region have, in the past examined a wide range of facets related to this pollution.

The marine and estuarine environment.

The presence of Avonmouth close to the Severn estuary has caused the the estuary to be contaminated with metals, although there are other smaller inputs from around the estuary. The distribution of metals within the estuary was described by Butterworth *et al*. (1972) and Nickless *et al*. (1972). Studies have been undertaken on the heavy metals in the fish in the estuary (Hardisty *et al*. 1974), shore isopods (Hopkin *et al*. 1985a) and amphipods (Pyke 1987). A more detailed analysis was carried out by Beckett (1986) who reviewed previous work and investigated metal levels in three coastal habitats, salt marshes, mudflats and rocky shores. The marine and estuarine environment are not the concern of this thesis and will not be discussed further.

2.2c *The terrestrial environment*.

The soils around Avonmouth have been shown to be highly contaminated with lead, cadmium and zinc (Burkitt *et al*. 1972, Little 1974, Little & Martin 1972). These elevated levels have been found to occur in vegetation in the area (Martin *et al*. 1980) although much of this metal is in the form of particles deposited onto the leaves (Little 1973), up to 80% of the lead, for example being accounted for by this (Little & Martin 1972). The relationship between metal concentrations in the soil and in the vegetation was explored in greater detail by Martin *et al*. (1982). Cadmium tolerant strains of the grass *Holcus lanatus* were found (Coughtrey & Martin 1977a, Coughtrey & Martin 1978a) which were discovered to arise quickly within the population (Coughtrey *et al*. 1978a). The distribution of cadmium within the grass was established (Coughtrey & Martin 1978b). The relationships between levels of pollution in the atmosphere from Avonmouth, and in the soil and various types of vegetation close by are described in more detail by Little (1974), Manning (1978) and Coughtrey (1978) the latter studied some invertebrates too.

Some of the woodlands in the area were found to have deep leaf litter layers which were the result of 25-30 years of litter input (Coughtrey et al. 1979). The numbers of fungi and bacteria were not found to be affected by the metal pollution (Martin et al. 1978, Martin et al. 1980). Therefore the numbers of invertebrate decomposers were considered to be the important group showing decreased activity (Coughtrey et al. 1978b). This was examined in a little more detail by Jones (1977).

Metal concentrations within invertebrate decomposers have been studied and experimented with (for example snails (Coughtrey & Martin 1976) and worms (Burkinshaw 1978, Wright & Stringer 1980)). Woodlice in particular were shown to accummulate large amounts of metals (Coughtrey et al. 1977) and have since been examined in more detail. It has been established that the most important storage organ for metals is the hepatopancreas (Hopkin & Martin 1982a) and within this in granules (Hopkin & Martin 1982b). Experiments using woodlice from Avon woods have been carried out (Coughtrey et al. 1980, Hopkin & Martin 1984a). Also animals from Avon compared with those from the Lizard in Cornwall, which provides a different group of metals for potential accumulation (Gent 1984).

As well as the decomposers, various predators have been examined for metal concentrations. In a series of experiments woodlice were fed to the spider *Dysdera crocata* (Hopkin & Martin 1985). Woodlice were also dissected and fed to the centipede *Lithobius variegatus* (Hopkin & Martin 1983, Hopkin & Martin 1984b, Hopkin *et al*. 1985b).

The passage of metals up the food chain has been considered many times. Martin & Coughtrey (1975, 1976) investigated several soil/litter feeding animals, whilst Henman (1981) and Hopkinson (1986) continued up the food chain to the predators as well.

Many of the publications have briefly considered the numbers of animals in the trophic levels in some of the woods. Only one study (Mould 1983) had investigated this in more detail prior to the commencement of the present study.

It is of interest that various methods for monitoring pollution levels have been established and used at Bristol. Moss bags (Little & Martin 1974) were used to monitor deposition rates. Using this method Gill *et al*. (1975) produced maps which could be related to various climatic data. Woodlice were suggested to be possible indicators by Martin *et al*. (1976) and have since been used as such by

Hardisty (1984) and Hopkin et al. (1986). Snails were also considered, however the relationship between metal concentration and weight causes problems (Coughtrey & Martin 1977b). Martin & Coughtrey (1982) summarise these methods of biomonitoring and others. In the present study one of these methods (moss bags) has been used further.

The pollution situation, particularly in some of the woods close to Avonmouth, has been widely studied for a long period of time. This has enabled studies to make comparisons over the years. For example Martin & Coughtrey (1987) have shown recent changes in metal concentrations in the soil with depth. Thus much of the background to the present work is fairly well established.

2.3 The effect of heavy metal pollution on invertebrates.

A surprising number of reviews relating to pollution in ecosystems appear to concentrate mainly on the soil and plants and neglect the heterotrophs. These last are often relegated to a paragraph or two, almost as an after thought, as grazers of the plants.

It is generally considered that a heavily polluted community will be lacking in numbers and diversity of organisms. Sensitive organisms will be reduced in number and those which are tolerant will thrive and dominate (Bordeau & Treshow 1978). This may favour the generalists, examples of which, given by Woodwell (1970) included gulls and rats, but might equally well be particular invertebrates. High doses of pollutants are thought to bring succession back to a previous stage at a lower diversity (Bordeau & Treshow 1978), though this assumes that earlier successional stages are lower in diversity. It is also reported that carnivorous animals are affected more than herbivores (Woodwell 1970).

Hughes *et al.* (1980) considered two categories of studies relating to heavy metal pollution on soil ecosystems. First studies relating to effects on soil processes and secondly those of changes in community structure and population dynamics. To this may be added a third category which I would consider perhaps the most frequently studied, that of determination of metal concentration within organisms and the passage of metals along simple food chains. It is this last area to which much of the literature is concerned, often concentrating on one link only of the chain. Perhaps the fact that man may be one of the last links has fuelled this impetus. Studies on changes in community composition has been sadly lacking from the literature although more recently moves to over come this are being made (Bengtsson & Rundgren 1984, Santas 1986). Perhaps the difficulty of analysing community structure and the problem of determining that any differences found are due to pollution has something to do with this paucity of data. The use of invertebrates as biological indicators has heightened general awareness, however, at a recent European workshop only three of the countries represented reported active interaction and funding from their government for studies of heavy metal pollution involving invertebrates. These did not include Britain (van Capelleveen & van Straalen 1986).

Wieser in 1966 published a much quoted paper on copper in isopods and since then a variety of work has been reported involving invertebrates and heavy metals. Today the isopod is still a favourite experimental animal. Many of the papers most pertinent to the present study are tabulated in Table 2.1. It can be seen from this that very few workers have been concerned with the density or diversity of the animals. Several sites of pollution have been well studied and in each, the papers produced have followed a predictable

Table 2.1

<u>SELECTIVE SURVEY OF LITERATURE PERTAINING TO HEAVY METALS (PRINCIPALLY Cd, Cu, Pb & Zn)</u>

<u>IN ECOSYSTEMS</u>

Emphasis is placed on invertebrates and site of particular interest (see text).

Literature with direct relevance to earthworms, collembola and mites, or small mammals is reviewed in the appropriate sections. Bristol-based work is also reviewed separately.

AUTHOR	SITE	COUNTRY	SOURCE OF POLLUTION	SUBJECT OF STUDY	COMMENTS
Roberts & Johnson 1978	N. Wales	Britain	Mine	I,M,V	)
Roberts *et al* 1978	N. Wales	Britain	Mine	S,V,M,I	) Invertebrates and
Roberts *et al* 1979	N. Wales	Britain	Mine	I,M,	) vegetation as food
Andrews *et al* 1984		Britain	Mine	I,M,	) for small mammals.
Andrews & Cooke 1984		Britain	Mine	I,M,	)
Williamson & Evans 1972	Durham	Britain	Road	I,M,	Pb Conc, in mammals & food
Williamson 1979	Durham	Britain	Road	Isopods	Conc. factors
Williamson & Evans 1972	Durham	Britain	Road	I	Abundance & lead conc.
Wade *et al* 1980	Scotland	Britain	Road	I	conc. & trophic level
Giles *et al* 1973	Maryland	U.S.A.	Road	I,V,	conc. related to food
Santas 1986	Washington DC	U.S.A.	Urban Area	I	diversity related to urbanisation
Clausen 1984		Denmark	General	Spiders	Pb in spiders

Table 2.1 contd

AUTHOR	SITE	COUNTRY	SOURCE OF POLLUTION	SUBJECT OF STUDY	COMMENTS
Gilbert 1971	Newcastle	Britain	General	I	Diversity in bark invertebrates
Maurer 1974		Switzerland	Road	Beetles spiders	diversity & numbers
Carter 1983		Canada	None	I	Cd related to food
Lepp 1979		Review			Cu in litter & decomposers
Alstad et al 1982		Review		I	air pollution & insects
Contescu & Hutchinson 1972	Sudbury	Canada	Smelter	V S	metals in vegetation & soils
Freedman & Hutchinson 1980[a]	Sudbury	Canada	Smelter	V	Diversity of plants
Freedman & Hutchinson 1980[b]	Sudbury	Canada	Smelter	VI	Decomposition, soil fauna
Munshower 1972, 1977	Deer Lodge Valley	U.S.A	Smelter	V I M A	Cd Values in grassland ecosystem movement or cd
Chaney et al 1978b	Chicago	U.S.A.	Urban	S V	) metals in soil &
Inman & Parker 1978	Chicago	U.S.A	Unrban	S V	) leaf litter effect) on decomposition
Parker et al. 1978	Chicago	U.S.A.	Urban	S V	conc. in soil & veget.
Van Hook et al. 1977	Missouri	U.S.A.	Smelter	S V	conc. & cycling
Van Hook 1974	Missouri	U.S.A.	Smelter	S worms	accumulation in worms
Van Hook & Yates 1975	Missouri	U.S.A.	Smelter	Spiders crickets	transfer in food chains

Table 2.1 contd

AUTHOR	SITE	COUNTRY	SOURCE OF POLLUTION	SUBJECT OF STUDY	COMMENTS
Watson *et al.* 1976	Missouri	U.S.A.	Smelter	I	Density, biomass, transfer
Jackson *et al.* 1978	Missouri	U.S.A.	Smelter	SV	Transfer of metals
Watson 1975	Missouri	U.S.A.	Smelter	S	conc in soil & decomp.
Boggess & Wixon 1979	Missouri	U.S.A.	Smelter	S V	transfer in ecosystem
Nash 1975	Palmerton	U.S.A.	Smelter	V	Lichen abundance & metals
Buchauer 1973	Palmerton	U.S.A.	Smelter	S V	Conc in soil & veget.
Jordan & Lechvalier 1975	Palmerton	U.S.A.	Smelter	Soil microbes	Numbers & metals
Beyer *et al.* 1984	Palmerton	U.S.A.	Smelter	Soil, isopods	Metals on isopods
Beyer 1986	Palmerton	U.S.A.	General	I	Biomagnification in food chain
Strojan 1978[a]	Palmerton	U.S.A.	Smelter	S I	Decomposition & invertebrates
Strojan 1978[b]	Palmerton	U.S.A.	Smelter	S I	Invertebrates & metal conc
Beyer *et al.* 1985	Palmerton	U.S.A	Smelter	I M	Invertebrates, mammal transfer
Fangmeier & Steubing 1986	Rhine	Germany	Industrial	I M V	conc. related to diet
Wieser 1966,'78,'79, Wieser *et al.* 1976			)) Labor.	Woodlice & snails	) Cu in decomposers)biological monitoring
Dallinger & Wieser 1977[abc]		Poland	Industrial	V I M S	Conc. transfer in ecosystem

Table 2.1 contd

AUTHOR	SITE	COUNTRY	SOURCE OF POLLUTION	SUBJECT OF STUDY	COMMENTS
Grodzinwski *et al.* 1984	Niepolomice	Poland	Industrial	V I M S	conc. transfer in ecosystem
Dmowski *et al.* 1979		Poland	Zinc Mill	S V I M	Conc. & Abundance
Lesniak 1980		Poland	General	Carabids	Abundance & dominance
Heliovaara *et al.* 1987	Harjavalta	Finland	Industrial	Insects	Concs. in insects
Van Capelleveen 1983		Holland	Steelworks	Isopods	Consump. & metal uptake
Joosse *et al.* 1983		Holland	Steelworks	Isopods	Tolerance
Joosse & Buker 1979		Holland	Laboratory	Collemb.	Excretion of lead
Van Straalen & Van Wensem 1986		Holland	Steelworks	I	Conc. related to body size etc.
Van Capelleveen *et al.* 1986		Holland	General	I	Invertebrates avoiding lead
Van Straalen *et al.* 1986		Holland	General	I	Conc. & Life Histories
Joosse & Verhoef 1987		Holland	Review	I	Metals & Animals
Hunter & Johnson 1982	Merseyside	Britain	Refinery	S V I M	Conc. in food chain
Hunter *et al.* 1984[a]	Merseyside	Britain	Refinery	S V I M	Conc. in food chain
Hunter *et al.* 1987[a]	Merseyside	Britain	Refinery	S V	Conc.
Hunter *et al.* 1987[b]	Merseyside	Britain	Refinery	I	Conc. & in food
Hunter *et al.* 1987[c]	Merseyside	Britain	Refinery	M	Conc. related to diet
Tyler 1975	Gusum	Sweden	Brass Mill	S	Conc. in soil & decomposition
Tyler 1972	Gusum	Sweden	Brass Mill	S V	Conc. in soil & veget.
Bengtsson & Rundgren 1982	Gusum	Sweden	Brass Mill	Enchytr-aids	Density and species

Table 2.1 contd

AUTHOR	SITE	COUNTRY	SOURCE OF POLLUTION	SUBJECT OF STUDY	COMMENTS
Bengtsson *et al.* 1983[a]	Gusum	Sweden	Brass Mill	Lumbricids	Conc. & Population
Bengtsson *et al.* 1983[b]	Gusum	Sweden	Brass Mill	Collemb.	Growth Rates & Metals
Bengtsson *et al.* 1985[a]	Gusum	Sweden	Brass Mill	Collemb.	Food sources & metals
Bengtsson *et al.* 1985[b]	Gusum	Sweden	Brass Mill	Collemb.	Reproduction & growth
Tyler 1984	Gusum	Sweden	Brass Mill	S V I	Review of metals in ecosystem
Bengtsson & Rundgren 1984	Gusum	Sweden	Brass Mill	I	Number of species & individuals
Sjögren 1986	Gusum	Sweden	Brass Mill	Collemb.	Competimon & metals
Bengtsson 1986	Gusum	Sweden	Brass Mill	I	Metals & invertebrates

I = Invertebrates. M = Mammals. V = Vegetation. S = Soil. Conc. = Concentration(s) pattern; soils, then vegetation, decomposition and micro-organisms, and finally invertebrates and mammals. In many situations invertebrates are considered solely as food for other animals (usually shrews).

There are a few centres of study which are worthy of further comment. The group headed by Prof. E.N.G. Joosse at the Free University, Amsterdam have been prolific in producing reports of the effects of heavy metals. Much of the work has a laboratory base and has involved Collembola in particular. More recently comments on behaviour and life histories in relation to pollution have been produced (van Capelleveen *et al*. 1986, van Straalen *et al*. 1986) as well as a report on metal concentrations in soil invertebrates discussed with reference to trophic level, body size and feeding mechanisms (van Straalen & van Wensem 1986).

Several areas of the United States of America have been studied, two detailed ones will be commented on here. The impact of the Palmerton smelter, Pennsylvania on the surrounding area has been recorded by Beyer, Strojan and co-workers. Strojan (1978b) studied the litter arthropods and recorded the abundance of various groups. Studies in the new lead belt in Missouri by Watson, van Hook and co-workers also included a report on forest litter arthropods including figures for densities and biomasses (Watson *et al*. 1976).

The effect of the brass mill at Gusum, Sweden has been reported by Tyler, Bengtsson and co-workers. Much attention here was paid to the invertebrates. Field work coupled with laboratory studies has lead to prediction of population survival of Collembola at polluted sites. Populations were also studied of enchytraeids (Bengtsson & Rundgren 1982) and lumbricids (Bengtsson et al. 1983a) and perhaps the most comprehensive study yet of invertebrate soil communities and heavy metal pollution (Bengtsson & Rundgren 1984).

More recently, work centred on Merseyside in Britain has reported on the flow of metals through a food chain (Hunter et al. 1987a,b,c). In contrast to many other areas of study this was a grassland site, and whilst much time was devoted to invertebrates they were considered mainly in order to determine metal concentrations in, and eventually as food for, mammals. Numbers were not reported for each site separately.

A few other studies are worthy of note here as they consider numbers and/or diversities of arthropods. Lesniak (1980) recorded changes in the structure of carabid communities with pollution. He showed that increase in pollution caused an increase in animals belonging to the dominant species and a decrease in individuals of rare species.

Williamsom & Evans (1972, 1973) examined the arthropod fauna at different distances from main roads. They concluded that habitat was more important in determining the numbers of animals present and that the results related to lead concentrations were not consistent between various groups; in many cases, animals were more abundant where lead concentrations were higher.

Santas (1986) recorded species diversity (H´, see section 7.4 for further details) in soil communities in relation to urbanisation. Diversity increased with distance from the centre of urbanisation and decreased with increased lead concentration of the soil.

Despite the difficulties of analysing community structures this study is an attempt to look at the effect of heavy metal pollution on the soil/litter arthropod communities, to identify any differences in diversity or numbers and highlight any particular species or groups of great relevance with regard to future pollution studies. One important point is that as many animals as possible have been identified down to species level. Many previous studies have neglected this point. It is known that similar species may differ widely in metal concentrations when collected from the same site and in sensitivity to metals (Eijackers in van Capelleveen & van Straalen 1986). Different behaviour patterns, habits and microhabitats between species are in my opinion frequently underrated.

Chapter 3.

METHODS.

3.1 ANALYSIS OF METAL CONCENTRATIONS.

3.1a Introduction to atomic absorption spectrophotometry. (AAS).

The method used for determining metal concentrations was atomic absorption spectrophotometry. This method is useful for a wide range of elements and can detect very low concentrations. Spectral interferences are also rarer than other methods. As the Varian Techtron handbook (1972) comments,

"Atomic absorption spectroscopy has now become the preferred technique for the analysis of complex mixtures."

The method measures the amount of energy atoms absorb to become excited from their ground state to a higher energy level. For any given path and wave lengths, the amount of energy absorbed is proportional to the concentration of the particular element concerned. As the relationship between absorption and concentration is linear over certain ranges of concentrations, by keeping path length and wavelength constant, the absorption can be compared to a series of standards. In the present study, a Varian Techtron AAS 775 was used, which has two modes of action, flame and flameless (graphite tube atomiser using a GTA 95). In both modes a background correction lamp was used, except for the analysis of copper, where the wavelength is above the maximum possible with the deuterium lamp used for background correction.

3.1b Flame atomisation.

In the flame mode, the sample is taken up in a continuous period of aspiration. The solution reaches the oxy-acetylene flame as a fine aerosol, where the thermal energy produces atomisation. Readings are taken at any point during the process of aspiration of the sample. This method is a relatively simple system which produces rapid results. It is good for samples with high concentrations of the element concerned and where a large volume is available (approximately 5ml per sample is required for the determination of each element). It is however relatively inefficient, only about 10% of the sample reaches the flame and the number of atoms used in taking the measurement is low in comparison to the number aspirated.

3.1c Graphite tube atomisation.

This is a method developed subsequent to flame atomisation, the full history of development is reported in Rothery (1982). For the purposes of the present study, the graphite tube atomiser has two main differences from the flame method. It is a more efficient system; for each measurement, a small volume (10μl in the present instance) is vaporised by rapid electrothermal heating and there is no wastage. Thus where only a small volume of sample is available this is a better method. Also, for samples containing much lower concentrations of metals it is the best method as the sensitivity is typically 100 times greater than that of the flame mode. The one main disadvantage is the increased length of time to obtain readings, particularly as there are more potential mechanical faults (e.g. with automated sample dispenser, tube aging etc.).

When using the graphite atomiser, the sample is introduced into the graphite tube though an aperture in the top. The tube is heated and three main stages can be identified (Culver 1975). First the drying stage, when smooth evaporation of the solvent from the sample is achieved. Secondly the ash stage, at an increased temperature which removes organic molecules and, ideally, leaves only the element required and a minimum of other compounds. Finally the atomisation stage when the temperature is raised rapidly to atomise all remaining compounds and measure the absorption. Programs controlling the heat required and rate of increase differ for each element and were modified from Rothery (1982).

3.1d Sample preparation and use of the machine.

Samples were all prepared by digesting in concentrated nitric acid. This is described by Rothery (1982) and used by other workers for example, Coughtrey (1978), Hopkin & Martin (1983, 1985) and Beckett (1986). Glassware was soaked in warm decontaminating fluid (2% DECON 90) for at least 24 hours, followed by 5 hours or more in 10% Analar grade nitric acid. It was then rinsed three times in deionised distilled water and dried in an oven.

Samples were also dried in an oven at 80^{o}C and soil and leaf litter samples were then ground to a powder in an pestle and mortar or an electric mill as appropriate. Accurately weighed aliquots of the samples (determined using an Oertling NA114 balance or a CI Miroforce balance) were placed into conical flasks or test tubes. A quantity of either Analar or Aristar grade concentrated nitric acid was added in proportion to the sample size and type (see Table 3.1 for details) and digestion then took place at boiling point on a hot plate. When digestion was complete and all the fumes of nitrogen IV oxide dissipated, samples were removed, cooled and made up to the appropriate volume (Table 3.1) using deionised distilled water. Any digests containing sediments were filtered using Whatman No. 1 filter papers. For each experiment, blank digests were also run. Table 3.1 also gives the mode of spectroscopy used for each type of sample to determine the metal concentrations. For samples containing concentrations of metals greater than the calibration standards, dilution with 20% analar or aristar nitric acid (usually in a ratio of 1 to 10) was carried out.

Whilst most types of interferences are not a problem when analysing lead, cadmium,copper and zinc from samples such as those taken in the present study, they were reduced further by employing certain precautions. A deuterium background correction lamp was used where possible to reduce non-atomic absorption. Both the standards and the samples were composed of the same acid matrix which was maintained even during dilution. This may not eliminate all matrix interferences but will certainly act to reduce them.

Because of the difference in sample sizes, dilutions undertaken and modes of action of the machine used, the various metals analysed have differing levels of detection for each sample. These detection levels are summarised in Table 3.2.

Table 3.1

TREATMENT OF DIFFERENT SAMPLE TYPES FOR METAL ANALYSIS

SAMPLE TYPE	APPROX. SAMPLE WT (g)	TREATMENT OF SAMPLE	GRADE OF HNO_3	VOLUME OF HNO_3 ADDED FOR DIGEST	ULTIMATE VOLUME	MODE FLAME (F) OR FLAME-LESS (FL)
MOSS BAGS	0.5	DRIED	ANALAR	10ml	50ml	F
Soil/leaf Litter	0.5	DRIED + POWDERED	ANALAR	10	50	F
Shrews (Whole + newts)	1.0	DRIED	ANALAR	10	50	F
Shrews (Livers + Kidney)	0.01 - 0.1	DRIED	ARISTAR	2	10	Zn F Rest FL
Glomeris/*Oniscus* Adult Millis/ Faeces	0.1	DRIED	ARISTAR	2	10	Both
Juv. Millis.	0.001 - 0.1	DRIED	ARISTAR	1	5	Zn F Rest FL
Worms	0.1	DRIED	ANALAR	2	10	F

Table 3.2

DETECTION LEVELS FOR THE DIFFERENT SAMPLES ANALYSED

Calculation incorporates detection limit of the machine, the sample size and the dilution factor.

F denotes flame mode FL denotes flameless

SAMPLE	Cd	Cu	Pb	Zn
Moss bags	0.01 F	0.1 F	0.1 F	0.1 F
Soil/Leaf Litter	0.1 F	0.1 F	1 F	0.1 F
Shrews (Whole) & Newts	0.01 F	0.1 F	1 F	0.1 F
Shrews (Livers & Kidneys)	0.005 FL	0.5 FL	0.005 FL	0.5 F
Glomeris, *Oniscus*, faeces - Adult millipedes	0.1 F	1 F	0.1 F	0.1 F
Juvenile Millipedes	0.01 F	0.1 F	0.1 F	0.1 F
Worms	0.01 F	0.1 F	0.1 F	0.1 F

3.2 METHODS OF CAPTURING WOODLAND ARTHROPODS.

3.2a. General.

In order to study the animal communities in woodlands, efficient methods of collection must be used. Obviously the method of sampling will vary according to the organism under observation. The same method cannot be used for invertebrates and mammals, for example. For ground living invertebrates, the most straight forward way of sampling is simply to sort by hand through the leaf litter and soil, however this is very time consuming, destructive if repeated frequently and in addition has been shown to be rather inefficient for many groups (Van Der Drift 1951). For smaller, less mobile groups, various extraction techniques can be employed to remove animals from small soil cores (see section 5.2). However for larger arthropods, which are perhaps more scarcely distributed another method is desirable.

Flying arthropods can be caught in many ways, for example light traps, colour traps or sticky traps. Invertebrates living amongst vegetation can be sampled by sweep netting or by suction machines. Specific species can be caught by special types of traps involving baiting or providing shelter. (Southwood 1980 provides a useful review or methods and apparatus). In order to sample a cross section of ground running woodland invertebrates, pitfall trapping is a good method. It is easier than sweep netting in areas of brambles, cheaper than suction machines, which are most useful for grassland habitats, and less selective, catching a broad spectrum of species. Pitfall trapping also has the advantages that captures can be made over a long period, with minimal time and energy spent sampling. There are many variations on the theme of pitfall trapping and the method has been heavily criticised, thus the intention of this section is to describe the method employed and to justify its use.

The pitfall trap is basically a glass or plastic container sunk into the ground. Much use has been made of 1lb jam jars (Briggs 1960, Mitchell 1963, Greenslade 1964) although many other shapes and materials have been tried. The material the trap is made of may affect the catching and retaining efficiency of the catch. Some species are able to avoid capture, for example the carabid <u>Nebria</u> <u>brevicollis</u> may be able to catch a hind claw over the lip of the trap and haul itself back up (Fairhurst 1969). If the surface of the trap is rough, the animals may be able to climb out, Luff (1975) showed that whilst glass jars prevented all escapes, the loss from plastic traps was 4% per day and from tin traps was 10%. The majority of escapes can be prevented by using a preservative (discussed later). The shape of the trap is usually round but may be square (Newton & Peck 1975) or gutter shaped, while Fairhurst (1969) considered the latter to be the most efficient shape, no account was made of the perimeter length or the trap mouth area. This last factor was shown to be the most important for the numbers of animals caught by Greenslade & Greenslade (1971). Gutter traps are not considered so useful for quantitative studies (Fairhurst 1969) therefore in the present study white plastic vending machine cups, 8cm in diameter and 11cm deep were used. The colour has been shown to have no effect (Greenslade 1964) and the main advantage of the cups was that, besides being light in weight to carry around, one could be positioned inside the other, so that a permanent position was established and minimum disturbance created at each collection time.

The positioning of the cups in the ground is critical and they were placed so that the lip was level with the soil surface. It was suggested by Greenslade (1964) that the vegetation around the traps should be cleared. This was not done in the present study as it would have been difficult in the circumstances, more plants emerging adjacent to the traps during the sampling period. Various workers have devised time sorting traps (Williams 1958, Desender et al. 1984) but in the present study it was not necessary to distinguish animals caught at different times of the day.

There has been much debate over the use of preservatives, some being thought to attract or repel the animals. Whilst Newton & Peck (1975) baited traps with carrion and dung or malt, for specific groups of beetles and Greenslade & Greenslade (1971) tried syrup and beer for certain ant species, traps generally contain a preservative or nothing.

Catching animals alive in traps may be required, for example for mark release recapture studies (Mitchell 1963) however this increases the chances of escapes and in-trap predation. An additional problem is that animals in traps not emptied frequently are subject to decay. In this study traps were planned to be emptied once a fortnight so a preservative was considered necessary. A wide variety of substances have been used for this purpose in the past, for example, potassium dicromate (Fairhurst 1969), ethylene glycol (Newton & Peck 1975), methylated spirits (Greenslade & Greenslade 1971), Gisin's fixing fluid (Joosse 1965), phenylmercuric acetate (Duffey 1962) and most commonly formalin (Ericson 1979, Wheater 1984, Baars 1979, Desender et al. 1984). A dilute formalin solution has the advantage that it is relatively cheap, does not discolour the specimens, as phenyl mercuric acetate does and does not evaporate quickly unlike alcohol based substances. Methylated spirits has been shown to attract various groups (Greenslade & Greenslade 1971), this may also occur with formalin solutions. Luff (1967) found increased numbers of some beetles in traps using 5% formalin, however Ericson 1979 reported no difference in numbers for the two species of Pterostichus he was studying. Skuhravy (1970) (cited by Ericson 1979) showed that formalin attracted female beetles more than males and Petruska (1969), (cited by Luff 1975) reported that small carabids and staphilinids of all sizes were still able to escape from traps containing formalin. Wheater (1984) compared catches of a wide variety of species groups in different strengths of formalin solution and in water; he found that only Diptera were affected. As Diptera were not explored in any detail in the present study, this is of little consequence. Thus it appears that whilst formalin may attract some species, this is probably true of all preservatives. Consequently a 4% formalin solution was used, containing a few drops of detergent to reduce surface tension. Traps were emptied every fortnight and a reasonable state of preservation was maintained throughout the study.

The presence of a lid over the traps helps to reduce potential predation by birds e.g. Magpies (Briggs 1960). This can also be stopped by providing shelter for the captured animals (Mitchell 1963) however the use of formalin should avoid this problem. Lids also stop vertebrates from succumbing to the traps but may restrict the capture of larger invertebrates e.g. beetles. Briggs (1960) notes that mesh roofed traps caught more animals than those covered by a solid disk. Joosse (1965) reports that roofs affect day active animals in particular and notes that,

"By putting a roof over a trap, a real intervention is made in the local environment. A change in several factors undoubtedly induces altered microclimatological circumstances under the roofs."

For these reasons and the low number of vertebrates caught at the commencement of the trapping, no roofs were provided. Unfortunately, later in the year some mammals were caught (see chapter 6).

The number of traps and the pattern of layout depends on the reason for trapping. Transects and single lines are helpful in examining zonation patterns (Hayes 1970) and causes less disturbance than a grid system (Fairhurst 1969). Having the traps in a close grid may cause a reduction in the potential catch, because animals on the path to one trap may fall into a previous one (Luff 1975). A grid system is best for quantitative studies (Fairhurst 1969, Baars 1979) and if placed regularly eases the problems of finding individual traps. Obrtel (1971) concluded that between 10 and 12 traps would be a sufficient number to collect information on the major species of Carabidae. Thomas & Sleeper (1977) suggest using uniform sized traps in a regular grid, collected at the same time intervals throughout the sampling period. They also suggest that continuous trapping in the same position may lead to overtrapping. This phenomenon was tested for (see appendix 2).

Bengtsson & Rundgren (1984) considered that when sizes and numbers of traps were consistent and duration of sampling was the same, data are comparable for different sites. Baars (1979) recommends that sampling should continue for a whole year using the same arrangement everywhere, in order to proceed with comparisons between sites. This was attempted in the present survey, however various other factors intervened, so strictly comparable data is available for a period of time which is shorter than a year (hereafter referred to as the standard trapping period). A digging-in period was also provided as suggested by Joosse (1965) who found that captures of some species were much higher at the commencement of trapping.

A set of pitfall traps can only sample from a relatively small area of a large habitat. Differences within one woodland can be investigated only by using several grids. Variation within the sites used for the present study undoubtedly occurs. Problems of this nature were reduced by selecting ´typical´ areas within the woods that were as superficially similar to the other sites selected.

Despite the problems of pitfalls, they have many advantages. Greenslade & Greenslade (1971) consider a large advantage is the simplicity and the ease of operation, certainly no special apparatus is required. Williams (1958) records that,

"Pitfall traps are shown to be sufficiently reliable, in the statistical sense, to reveal the differences in the number of animals caught at different times of the year, and in different habitats."

Different species will undoubtedly behave differently with respect to traps but other factors will be involved too, for example weather conditions (Ericson 1979), sex of the animal (Greenslade 1964) and life cycle stage (Hayes 1970). Density dependent dispersal (ie. a relationship between the density of a population and the movement of animals from it), which would be perhaps the most important in the present study is considered probably not to occur (Baars 1979). Attempts to measure absolute density levels from pitfall captures have been suggested by Fairhurst (1969) and Baars (1979), however, although this may be feasible for a few species it is not practicable for large numbers of diverse groups. It is important to remember at all times that captures are a result of activity as well as abundance, however by standardising methods in every place and collecting over a long period of time, comparisons can be made between the sites. Whilst any method of collecting will invariably miss some species and vary in selectivity for different species, it is still valid to compare those caught by a particular method, which is used in different sites.

3.2b Summary of methods used.

15 pitfall traps were laid at each site in a 5x3 grid. Each trap was set using a horticultural bulb planter, in a position that was 1m from its neighbours and consisted of two white plastic cups 8cm in diameter and 11cm deep, one

inside the other. The lip of the inner cup was flush with the surface of the ground and contained approximately 5ml of 4% formalin solution, with a few drops of detergent. At 14 day intervals the sites were visited in a standard order. The inner cup was removed, the contents were washed into a plastic bottle and a fresh formalin solution added. The cup was then replaced in the outer cup. The contents of the traps were taken to the laboratory, transferred into 70% alcohol and identified, where possible to species level. Flies were not identified, neither were mites and collembola which are treated in a separate section (see section 5.2).

Chapter 4.

SITE DESCRIPTIONS.

Site.1. General.

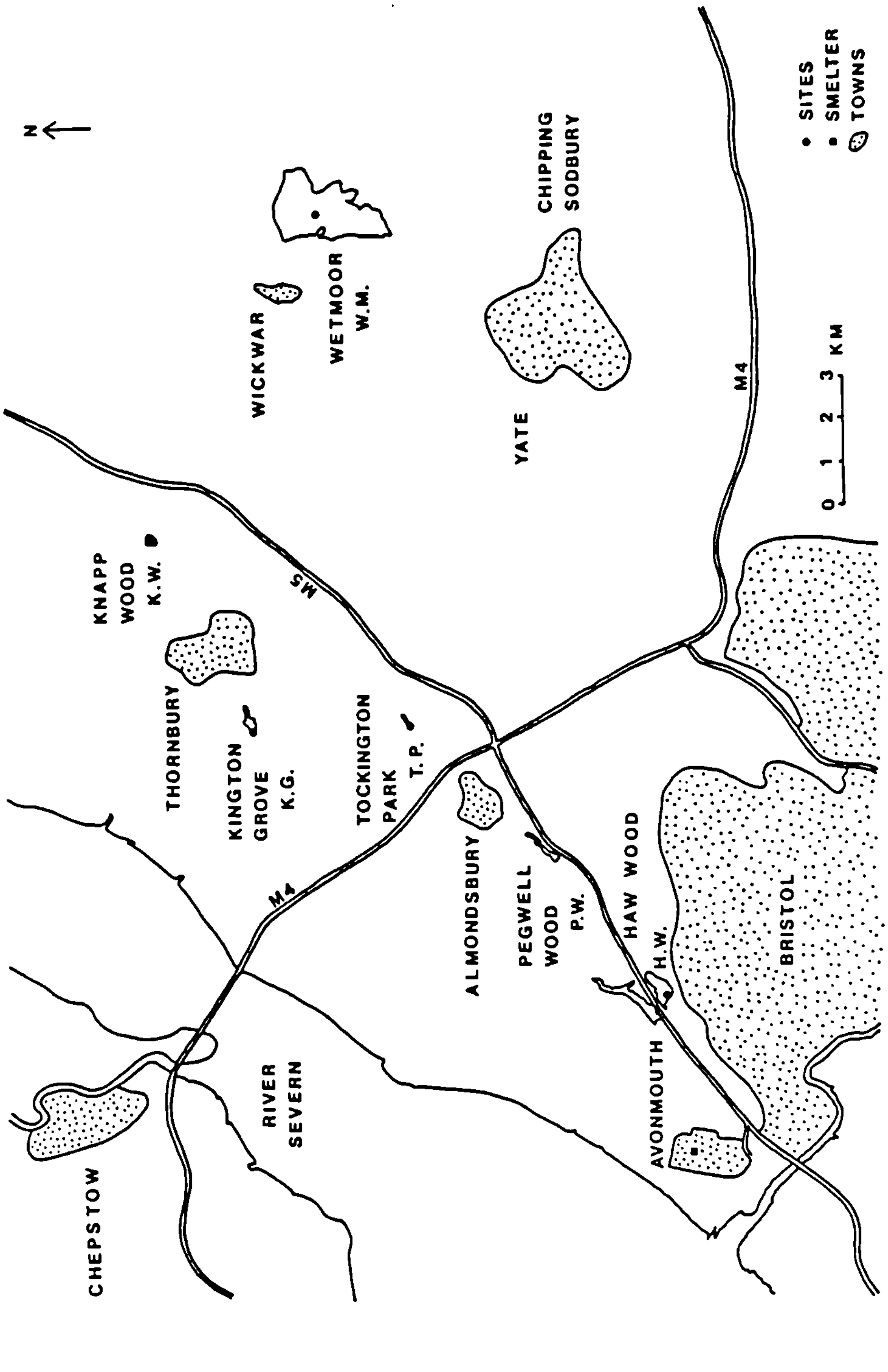
The sites chosen for sampling are all found along a line running approximately north eastwards from the smelting works at Avonmouth, as this is the direction of the prevailing winds (Gill *et al*. 1975) (see also section 4.2). Also running in this direction is the Tortworth ridge (Findlay 1976) which is a sharply defined area of higher land extending north easterly from Hallen and most of the sites are situated on this ridge. The ridge curves at Tortworth to continue southwards to Wickwar and Chipping Sodbury close to Wetmoor wood (see Figure 4.1).

Woodlands used for sampling were selected in order to be as similar as possible except in degree of pollution. Inevitably, the availability of woods, ease of obtaining owner´s permission and ready access and the necessity of selecting in the autumn, meant that differences invariably occurred as the seasons progressed. The locations of the sites are shown in Figure 4.1. All the sites were mixed deciduous woods containing oak and ash with a thick layer of brambles. A more detailed vegetation survey is given in section 4.3.

Figure 4.1 Map to show the location of the sampling sites.

Throughout the thesis sites are refered to by name and number:-

1 Wetmoor	4 Tockington Park
2 Knapp Wood	5 Pegwell wood
3 Kington Grove	6 Haw wood (Most polluted site)


N
SITES
SMELTER
TOWNS
CHIPPING SODBURY
WICKWAR
WETMOOR
W.M.
YATE
M4
0 1 2 3 KM
KNAPP WOOD
K.W.
M5
THORNBURY
KINGTON GROVE
K.G.
TOCKINGTON PARK
T.P.
ALMONDSBURY
PEGWELL WOOD
P.W.
HAW WOOD
H.W.
BRISTOL
M4
AVONMOUTH
RIVER SEVERN
CHEPSTOW

General details of the sites are given in Table 4.1. The distances, altitude and area measurements are taken from the Ordnance survey 1:50 000 map number 172.

The soil type of each site was established using the Soil Survey of England and Wales map sheet 5. The following descriptions of the relevant soil associations are taken from Findlay et al. (1984).

Wetmoor, Tockington Park and Haw wood are all on the Denchworth series. This is a stoneless, mottled clayey soil which is waterlogged for much of the winter, clearly seen at Wetmoor. This soil type is best used for dairy and permanent grassland which can be seen in the areas of Tockington Park and Haw wood.

Knapp wood and Pegwell wood are on Worcester series. Another clayey soil, this is rather more reddish in colour and may have a thin layer of fine loamy soil on top. Also used as permanent grassland as it becomes seasonally waterlogged; this soil will also grow cereals particularly winter sown ones, as seen adjacent to Knapp wood.

The remaining site, Kington Grove, according to the soil survey map is on the junction of Worcester and Hodnet series. Due to the more sandy nature of the soil and the presence of bracken, it seems more likely to be considered

Table 4.1

SUMMARY OF DETAIL ABOUT THE SITE

	HAW WOOD	PEGWELL WOOD	TOCKINGTON PARK	KINGTON GROVE	KNAPP WOOD	WETMOOR WOOD
GRID REFERENCE (ALL ST)	558 800	593 826	624 858	622 893	665 916	743 874
DISTANCE FROM SMELTER (KM)	3.0	7.8	11.6	13.9	18.8	23.0
DISTANCE FROM SEA (KM)	3.4	5.6	6.4	3.75	6.55	15.25
ALTITUDE (M)	65	70	80	75	65	75
AREA OF WOOD (KM^2)	0.92	0.35	0.08	0.26	0.04	3.82
SOIL TYPE	Denchworth	Worcester	Denchworth	Hodnet	Worcester	Denchworth
LITTER DEPTH (MM)	131 ± 23.8^{c}	85.6 ± 16.3^{b}	44 ± 12.9^{a}	86 ± 20.4^{b}	61 ± 1.04^{ab}	32 ± 13.96^{a}

Results of analysis of variance for litter depth, $F = 18.55$ df 5/24 $p<0.01$**

Results of subsequent fixed range test are shown in the table. Any two means with the same letter as a superscript are not significantly different.

as Hodnet series (Bullock pers. comm.). A deeper loamy reddish soil, subject to slight seasonal waterlogging, the Hodnet series will support cereals as well as pastureland. To conclude comments on the soil types, it seems that a gradient of factors from heavy clay, waterlogged areas to more sandy, better drained conditions can be identified in the woodlands selected.

In January 1986 soil and leaf litter samples were taken from the sites for metal analysis and pH evaluation. Three layers were taken from each site; the leaf litter, a mixture of the fermentation and humus layers and the mineral soil. 15 replicates of each layer were taken and analysed for cadmium, copper, lead and zinc. The results are illustrated in Figures 4.2 a-d. The graphs show the characteristic exponential decrease in metal concentrations moving away from the source of pollution (Munshower 1972, Hunter & Johnson 1982). A non-parametric test of concordance (Meddis 1984) shows the same degree of change of metal concentration throughout the sites, ($H=19.4$, $df=5$, $p=0.002$). A two way analysis of variance was calculated for each metal to test for differences between the sites and the three soil layers. In every case both factors were shown to have a significant effect. Because the effect of depth over all the sites is not of interest, this was not pursued further. The mean values for each site however were examined by use of a fixed range test (Parker 1979), the results are shown in Table 4.2. It can be seen that Haw wood is predominantly

Figure 4.2 Graphs showing the concentrations of metals recorded from each site, from each of three soil layers.

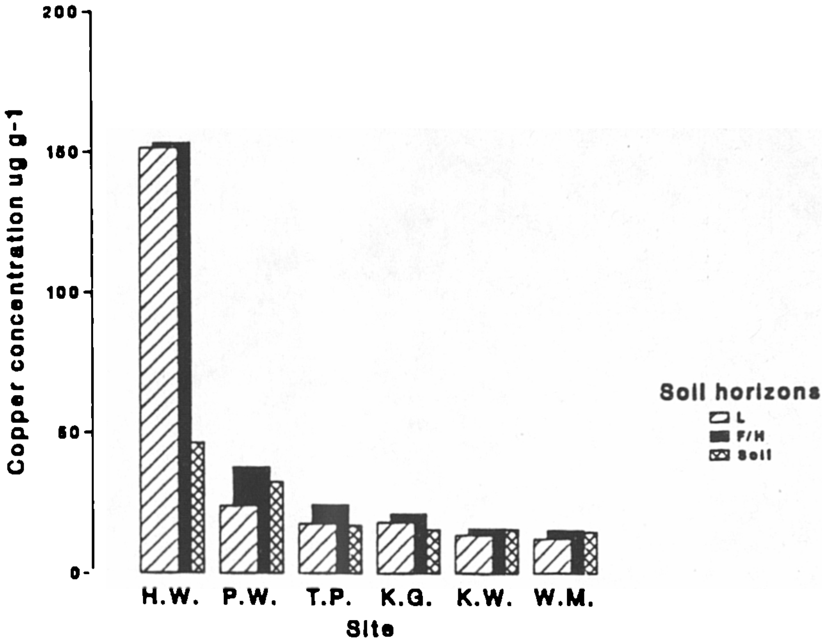
4.2a Copper

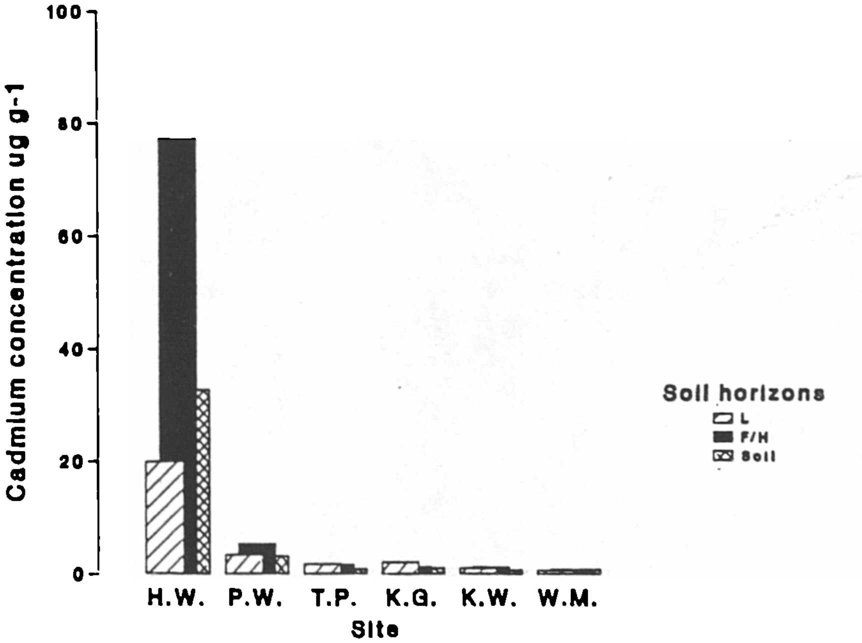
4.2b Cadmium

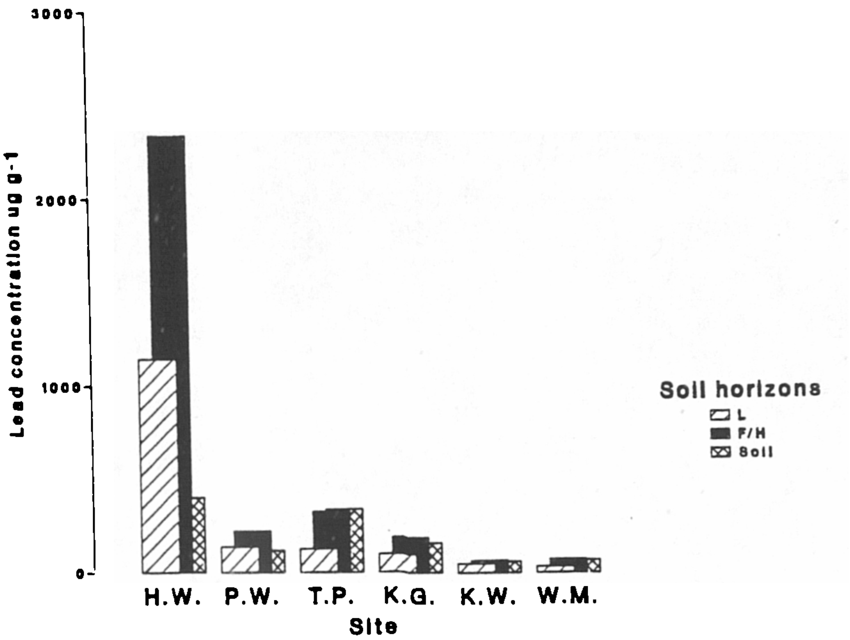
On following page:

4.2c Lead

4.2d Zinc


200
150
100
50
0
Copper concentration ug g-1
H.W.
P.W.
T.P.
K.G.
K.W.
W.M.
Site
Soil horizons
L
F/H
Soil


100
80
60
40
20
0
Cadmium concentration ug g-1
H.W.
P.W.
T.P.
K.G.
K.W.
W.M.
Site
Soil horizons
L
F/H
Soil


3000
2000
1000
0
Lead concentration ug g-1
Soil horizons
L
F/H
Soil
H.W. P.W. T.P. K.G. K.W. W.M.
Site

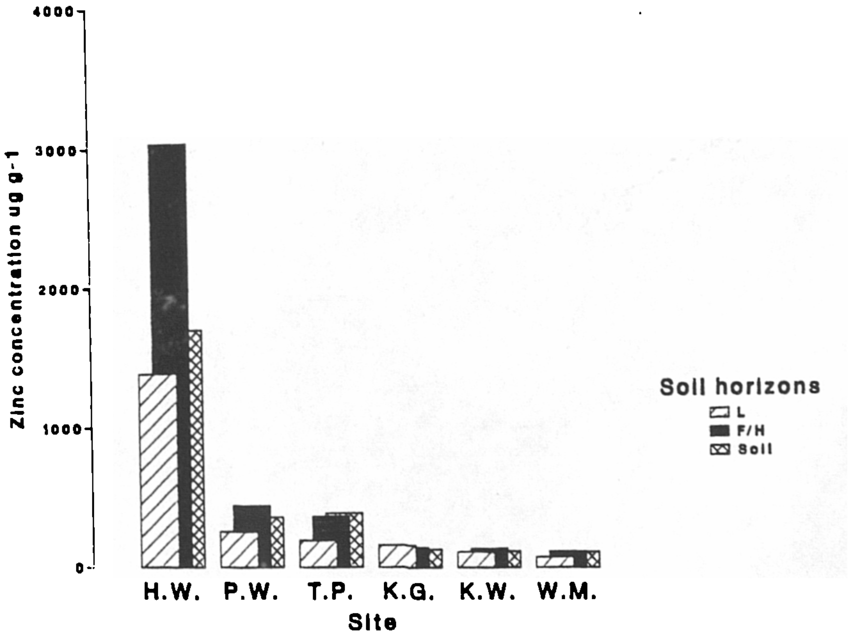

4000
3000
2000
1000
0
Zinc concentration ug g-1
Soil horizons
L
F/H
Soil
H.W. P.W. T.P. K.G. K.W. W.M.
Site

Table 4.2

METAL CONCENTRATIONS PER SITE AT EACH OF 3 DEPTHS (MEAN ± STANDARD ERROR)

METAL/ DEPTH	HAW WOOD	PEGWELL WOOD	TOCKING-TON PARK	KINGTON GROVE	KNAPP WOOD	WETMOOR	F	DF	P
CADMIUM A	19.88^{ab} ±1.3	3.3^{b} ±0.147	1.68^{ab} ±0.06	2.04^{ab} ±0.089	1.01^{ab} ±0.044	0.604^{a} ±0.0556	52.16	5/252	<0.01
B	77.24^{d} ±6.25	5.2^{bc} ±0.45	1.589^{a} ±0.08	1.21^{ac} ±0.12	1.17^{ab} ±0.056	0.804^{ad} ±0.05			
C	32.64^{c} ±3.75	3.06^{b} ±0.39	0.77^{a} ±0.069	0.98^{ab} ±0.065	0.66^{a} ±0.082	0.775^{a} ±0.056			
TOTAL	43.25^{c} ±4.42	3.85^{b} ±0.25	1.34^{ab} ±0.07	1.41^{ab} ±0.09	0.95^{ab} ±0.05	0.73^{a} ±0.03			
COPPER A	151.41^{b} ±9.52	23.93^{a} ±0.83	17.64^{a} ±0.57	18.17^{a} ±0.56	13.68^{a} ±0.41	12.26^{a} ±0.44	119.9	5/252	<0.01
B	153.16^{b} ±5.16	37.75^{a} ±1.09	24.33^{a} ±0.77	21.22^{a} ±0.92	15.96^{a} ±0.44	15.23^{a} ±0.34			
C	46.38^{b} ±3.35	32.54^{ab} ±0.79	17.11^{a} ±0.77	15.56^{a} ±0.56	15.72^{a} ±0.71	14.69^{a} ±0.44			
TOTAL	116.98^{b} ±8.38	31.41^{a} ±1.0	19.69^{a} ±0.64	18.32^{a} ±0.53	15.12^{a} ±0.34	14.06 ±0.3			
LEAD A	1142.4^{b} ±66.3	137.94^{c} ±5.89	125.67^{ac} ±7.28	97.95^{ac} ±5.84	49.98^{ac} ±2.16	32.84^{a} ±2.39	114.0	5/252	<0.01
B	2340^{b} ±104	219.5^{ac} ±17.5	323.2^{c} ±21.1	186.4^{ac} ±16.4	66.32^{a} ±2.27	70.88^{a} ±1.38			
C	398.2^{b} ±48.6	116.8^{ac} ±12.8	335.5^{bc} ±31.4	159.2^{bc} 37.5	60.33^{a} ±3.04	66.81^{a} ±1.9			

Table 4.2 contd

METAL/ DEPTH	HAW WOOD	PEGWELL WOOD	TOCKING-TON PARK	KINGTON GROVE	KNAPP WOOD	WETMOOR	F	DF	P
TOTAL	1294^{b} ± 128	158.1^{c} ± 9.9	261.4^{ac} ± 19.2	147.8^{ac} ± 14.6	58.9^{a} ± 1.75	56.84^{a} ± 2.8			
ZINC A	1389.8^{b} ± 75.5	257.0^{ab} ± 11.1	193.8^{ab} ± 10.4	159.17^{ab} ± 4.33	112.84^{ab}. ± 5.37	74.45^{a} ± 3.07	102.9	5/252	< 0.01
B	3039^{b} ± 252	442.2^{ab} ± 19.7	366.2^{ab} ± 24.8	142.57^{a} ± 5.6	136.32^{a} ± 3.29	114.34^{a} ± 2.39			
C	1704^{a} ± 105	360.9^{a} ± 17.4	390.1^{c} ± 25.8	129.72^{a} ± 6.25	116.04^{a} ± 4.96	111.17^{a} ± 2.85			
TOTAL	2044^{b} ± 142	353.4^{ab} ± 14.7	316.7^{ab} ± 17.9	143.82^{ab} ± 3.6	121.73^{a} ± 3.05	99.98^{a} ± 3.15			

F Values are results of between site ratios from two way analysis of variance. Superscripts indicate results of subsequent fixed range tests. Two means showing the same letter are not significantly different from each other. A = Litter. B = F/H Layer. C = Mineral Soil.

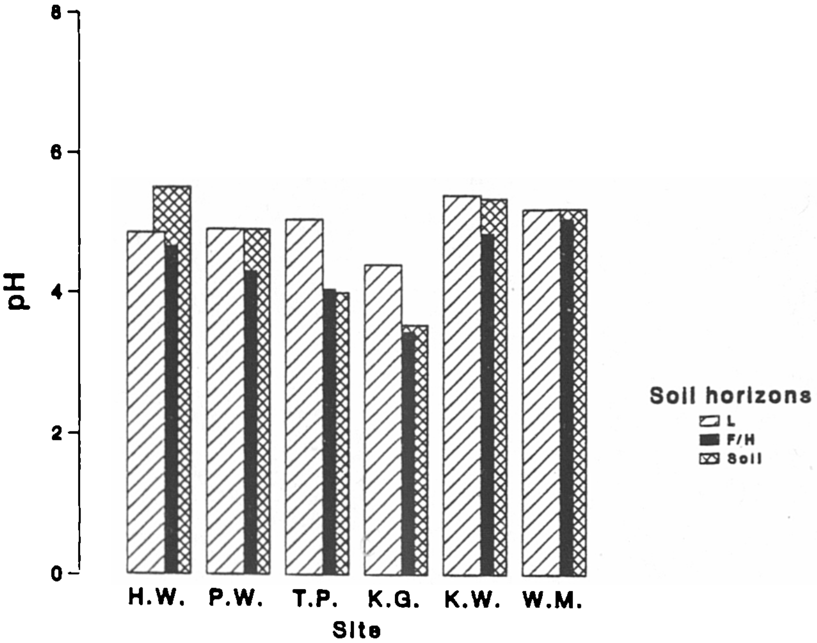
different from all the other woods except (in a few cases) Pegwell wood. In general the concentration of metals is greatest in the F/H layer at all the sites and lowest in the mineral soil.

Two replicates of each layer were taken for pH determination. One part sample to 2.5 parts deionised distilled water were mixed together and the pH recorded using an Electronic Instruments Ltd. pH meter 7020. The results are illustrated in Figure 4.3. Using the data for all three layers at each site a twoway analysis of variance showed significant differences existed between the sites (Table 4.3). Computation of fixed range tests show that Kington Grove is frequently significantly more acidic than the other sites, particularly Knapp wood, Wetmoor and Haw wood.

In March 1986 the depth of leaf litter was measured at five random points close to the trapping grid. The data are shown in Table 4.1 and Figure 4.4. It can be seen that Haw wood, the most contaminated has by far the deepest litter layer. Oneway analysis of variance showed the differences between the sites to be significant ($F=18.55$, $p<0.01$); subsequent calculation of a fixed range test (Parker 1979) shows Haw to have a significantly greater depth of litter than the other sites (see Table 4.1 for details). In view of the fact that both litter depth and pH vary between the sites, as well as the metal concentrations, both of these

Figure 4.3 Graph to show pH in each soil layer at each site.

Figure 4.4 Graph to show mean litter depth at each site.


pH
8
6
4
2
0
H.W.
P.W.
T.P.
K.G.
K.W.
W.M.
Site
Soil horizons
L
F/H
Soil

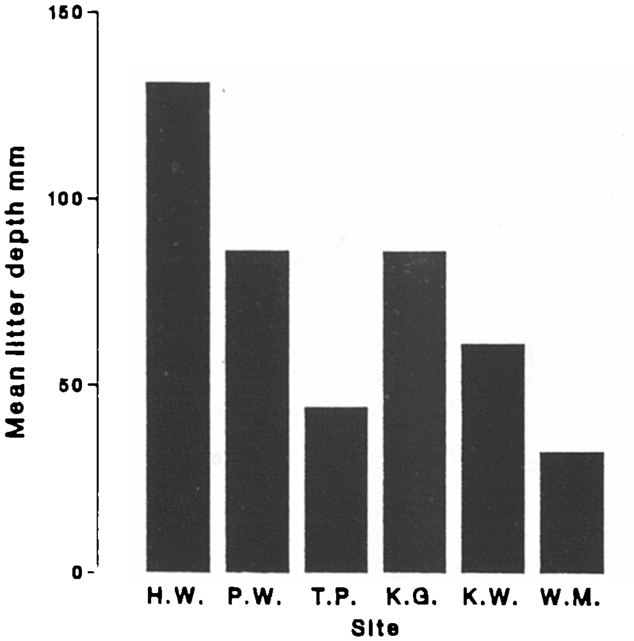

Mean litter depth mm
150
100
50
0
H.W.
P.W.
T.P.
K.G.
K.W.
W.M.
Site

Table 4.3

pH VALUES FOR EACH SITE AT DIFFERENT DEPTHS (Mean $\pm$ Standard Error)

DEPTH	HAW WOOD	PEGWELL WOOD	TOCKING-TON PARK	KINGTON GROVE	KNAPP WOOD	WETMOOR WOOD	F	df	p
A	4.85 ± 0.05^{a}	4.9 ± 0.2^{a}	5.05 ± 0.35^{a}	4.4 ± 0.6^{a}	5.4 ± 0.0^{a}	5.2 ± 0.0^{a}			
B	4.65 ± 0.05^{ab}	4.3 ± 0.4^{ab}	4.05 ± 0.85^{ab}	3.45 ± 0.05^{b}	4.85 ± 0.15^{a}	5.05 ± 0.25^{a}	9.041	5/18	<0.01
C	5.5 ± 0.01^{a}	4.9 ± 0.1^{ab}	4.0 ± 0.4^{b}	3.55 ± 0.25^{b}	5.35 ± 0.15^{a}	5.2 ± 0.1^{ab}			
TOTAL	5.0 ± 0.165^{a}	4.7 ± 0.173^{ab}	4.37 ± 0.34^{ab}	3.8 ± 0.25^{b}	5.2 ± 0.124^{a}	5.15 ± 0.08^{a}			

F Value is result of between site ratio from two way analysis of variance.

A = Litter. B = F/H Layer. C = Mineral Soil.

Superscripts show results of subsequent fixed range tests. Two means showing the same letter are not significantly different from each other.

factors have been used in further statistical analyses. Although build up of litter occurs in regions of high pollution (e.g. Tyler 1972) this is not the sole cause of deep leaf litter as the site at Kington grove illustrates (see Table 4.1).

4.2. Climate.

The climate is potentially an important factor in determining which organisms are found at a particular site. Any differences between the sampling sites could be of great significance when examining the community structure. Unfortunately, weather stations are sparsely located and not all release comprehensive data. Long Ashton weather station is to the south west of the sites, and Cheltenham weather station is to the north. Mean monthly maximum and minimum temperatures and the total monthly rainfall and sunshine hours were all compared using t-tests and found to be not significantly different ($p>0.05$) see table in appendix 2 for details. This suggests that there may be little variation in climate between the sites sampled in. As Long Ashton weather station is the nearest to the sites which issues comprehensive data, information from there was used in future statistical analyses. Weather data during the period of trapping is shown in Table 4.4. Daily details were combined into trapping collections.

Wind speeds and directions were available from Avonmouth harbour masters office and data over several years were used

Table 4.4

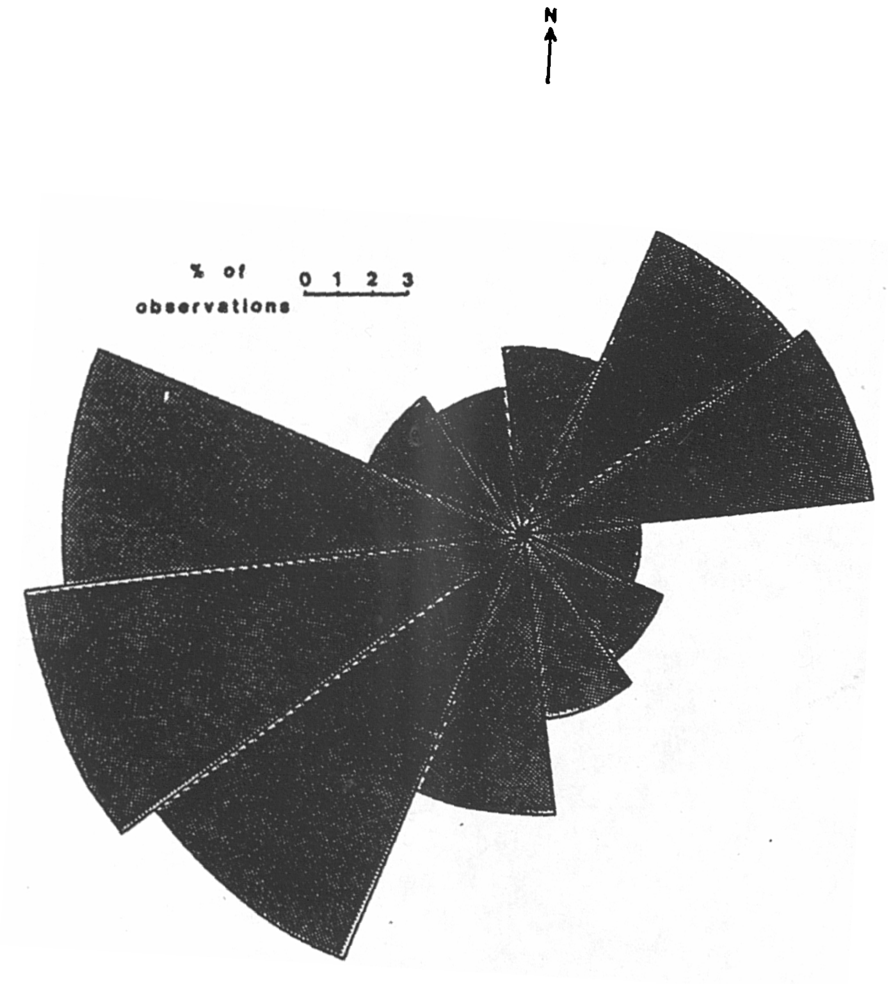
WEATHER DATA FROM LONG ASHTON SHOWN AS PER TRAPPING OCCASION

Collection		No. of Frosts	Mean Max. Temp °C	Mean Min. Temp °C	Total Rainfall (mm)	Mean Rainfall (mm)	Total Sunshine (hours)	Mean Sunshine (hours)	Mean Soil Temp at 10cm °C
Date	No								
1985									
2-1	1	3	8.4	2.4	24.5	1.75	12.5	0.9	-
23-1	2	19	2.4	-3.4	35.9	1.71	38.2	1.8	-
31-1	3	5	8.9	1.7	33.6	4.2	4.5	0.6	-
28-2	4	9	7.2	1.7	85.1	2.36	88.3	2.5	-
4-3	5	3	10.4	2.4	8.8	0.63	60.4	4.3	4.6
28-3	6	8	7.1	-0.5	25.5	1.8	51.3	3.7	2.9
11-4	7	0	13.0	6.9	62.3	4.45	37.5	2.7	8.3
25-4	8	1	13.1	3.7	7.6	0.5	91.6	6.5	8.0
9-5	9	1	14.0	4.6	12.8	0.9	103.1	7.4	9.8
23-5	10	0	15.2	7.1	33.2	2.37	38.5	2.7	11.3
6-6	11	0	18.6	9.0	47.1	3.36	129.9	9.3	14.8
20-6	12	0	16.3	6.9	44.9	3.21	93.6	6.7	13.6
4-7	13	0	18.9	11.5	61.7	4.4	74.1	5.3	15.6
18-7	14	0	20.6	11.7	19.7	1.4	103.6	7.4	18.0
1-8	15	0	20.0	12.1	58.3	4.2	78.9	5.6	16.6
15-8	16	0	18.1	11.6	80.2	5.7	77.2	5.5	15.1
29-8	17	0	18.7	12.0	49.5	3.5	85.1	6.1	15.3
12-9	18	0	18.9	9.9	23.8	1.7	84.6	6.0	14.1
26-9	19	0	18.6	11.5	9.9	0.7	42.4	3.0	14.5
10-10	20	0	18.9	11.7	53.3	3.8	55.9	4.0	13.7
24-10	21	0	13.8	7.3	0.2	0.01	39.3	2.8	10.9
7-11	22	1	11.0	4.3	12.3	0.9	40.7	2.9	7.4

Table 4.4 contd

Collection Date	Collection No	No. of Frosts	Mean Max. Temp °C	Mean Min. Temp °C	Total Rainfall (mm)	Mean Rainfall (mm)	Total Sunshine (hours)	Mean Sunshine (hours)	Mean Soil Temp at 10cm °C
21-11	23	4	7.5	1.6	24.3	1.7	43.4	3.1	5.0
5-12	24	2	9.0	4.5	35.4	2.5	8.5	0.6	5.8
19-12	25	1	10.5	5.8	49.2	3.5	14.6	1.0	7.2
1986									
2-1	26	5	7.1	1.6	102.7	7.3	15.2	1.1	4.1
16-1	27	4	7.2	1.6	31.8	2.3	18.5	1.3	3.2

Figure 4.5 Wind rose to illustrate the distribution of wind directions. Data from Avonmouth harbour masters office for the last 10 years.


N
% of observations
0 1 2 3

to construct a wind rose Figure 4.5 (Avonmouth being much closer to the source of pollution than Long Ashton). This confirms that the prevailing winds blow in the direction of the sampling sites.

The site at Wetmoor is much further inland than the others and probably experiences slightly different weather conditions. No detailed information is available for this site, however Findlay et al. (1984) record that with increasing distance from the sea, a shift from a maritime dominated climate towards a more continental one occurs. This leads to a drop in rainfall and a decrease of temperatures especially in winter. This is consistent with points noted while undertaking field work. Wetmoor also received more snow which persisted longer in the winter trapping periods.

4.3. Vegetation.

4.3a. Introduction.

Tansley in 1939 discussed and described British woodlands at length. In 1911 he had classified different types of wood according to the dominant tree species present, for example, beech, alder or sessile oak, and the soil type. In this classification the majority of the sites in the present study fall into the category of pedunculate oak woods or *Quercetum roboris*. The sole exception being Knapp wood, which supports sessile oaks (although they are undoubtedly planted), hence perhaps *Quercetum petraeae*. Within the two branches of oak woodlands, Tansley identified different field layer societies, for example that of Hyacinthoides non-scriptus (formerly Scilla), that of Rubus and Pteridium and that of Mercurialis perennis, all of which might be applicable to one of more of the sites sampled. Whilst Wetmoor with its oak standards and understorey of hazel coppice is perhaps an ideal 'Tansleyan' Quercetum roboris (damp oak wood), other trees, notably ash and field maple may be present. The Tansley system was produced at a time when many woodlands were actively managed or management had recently ceased. Today, the majority of woods including those in the present study have been left unmanaged for a period of time. This has led to changes in structure and species composition so that the classification categories of Tansley are of less use.

In 1977 habitats were classified for use by the Nature Conservancy Council by Ratcliffe. The break down of woodlands was done according to dominant tree type and region of Britain. Whilst mixed deciduous woods are considered, much of the emphasis was still on one or two dominant species.

More recently, three new classification methods have been proposed. First the Stand Type of Peterken described in detail in 'Woodland conservation and management' (1981), secondly the Merlewood Plot Type of Bunce (1982) and thirdly the National Vegetation Classification sponsored by the

Nature Conservancy Council. Of these three, only the first two were readily available and these will be discussed below.

Peterken´s stand type system is based on the tree and shrub species present in a 30 x 30m area. Details of the soil type are also used and a list of other vascular plants present given for each sub-type. A key to the stand types is given in Kirby (1984) taken from Peterken (1980).

The Merlewood plot type classification in contrast is based predominantly on ground vegetation. Species found in a $200m^2$ area are recorded and the key (Bunce 1982) works on the presence or absence of several indicator species at each stage.

Kirby (1984) compares the two methods and found that woods shown to be similar using one method were also classed as similar when the other method was used. This led him to conclude that the two methods are broadly equivalent, and he suggests that they may be complimentary in many ways.

4.3b Methods.

The vegetation at each site was surveyed on the 15th and 16th of July 1985. An area 30 x 30m was marked out which included in it the pitfall trapping grid (see section 3.2) and areas used for Tullgren samples (see section 5.2). In this area, the number of trees and shrubs of each species was recorded and a list made of vascular plants and bryophyte species present. Canopy cover was estimated for the area. In the region enclosed by the edges of the pitfall grid, three $1m^2$ quadrats were placed and percentage cover of each species present was recorded together with the average height of the vegetation.

4.3c Results.

The species recorded in the 30 x 30m area in each site are listed in appendix 4, along with the percentage cover of the three $1m^2$ quadrats for the sites. The latter give an indication of the important ground species in the area of the pitfall traps. Except for Pegwell wood which was dominated by *Geranium robertianum* the sites were clearly dominated by *Rubus fruticosus* sens. lat.. Analysis of variance on bramble cover show significant differences between the sites ($F=7.68$, $p<0.01$) employing a fixed range test it can be seen that Pegwell wood is significantly different from all the other sites except Haw wood. Haw is not significantly different from any of the other sites.

4.3d Classification of the sites.

The sites were first classified using Peterken's stand type system. The key was used to the main stand types, then descriptions (Peterken 1981) followed to obtain the final classification. In several instances the ultimate sub type could not be distinguished and the result is intermediate between two closely related alternatives. Results are summarised in Table 4.5.

Both Wetmoor and Haw wood keyed out at 2Aa 'typical' wet ash maple woods. Whilst Haw wood fitted the description fairly well, Wetmoor had some characters which are seen in 2Ab, most notably a lower frequency of ash.

Tockington Park was described well by 1Ba wet ash-wych elm. Knapp wood also fell into this category except that it had Quercus petraea in place of Q. robur which Peterken reported never to occur in 1Ba. Accordingly, Knapp wood could be seen as intermediate between 1Ba and 1D, the western valley ash wych-elm woods which is a Q. petraea association. However the ground flora did not fit 1D.

Kington Grove, keyed out at group 3 and was best identified as an intermediate between 3Aa and 3Ab, both acid pedunculate oak hazel ash woods. The species list for the heavy soil variety (3Aa) fitted quite well however 3Ab, although found on lighter soils, often has bramble and blue bell dominating the ground layer, as found at Kington Grove.

Pegwell wood keyed out as the outsider at 9A, pedunculate oak hornbeam stand. Although probably nearer to 9Ab the ash

Table 4.5

SUMMARY OF THE VEGETATION AT EACH SITE

	HAW WOOD	PEGWELL WOOD	TOCKING-TON PARK	KINGTON GROVE	KNAPP WOOD	WETMOOR WOOD	X^2
In 30 x 30m							
No. Trees Sp. (>½canopy)	5	6	4	4	4	5	
No. Shrub Sp. (<½canopy)	11	8	4	4	4	2	
No. Herb Sp.	28	29	31	24	35	35	3.01 ns
No. Bryophyte Sp.	3	2	4	2	0	8	
Total No. Sp.*	35	39	35	32	40	39	1.34 ns
Canopy Cover (%) †	40	60	30	30	40	60	21.54 ***
Density of Trees to Canopy (Standards/m^2)	0.02	0.03	0.018	0.011	0.027	0.038	
In quadrants ($1m^2$)							
Mean No. Vascular Sp.	4.3	7	7	5.3	6	7	1.04 ns
Mean No. Bryophyte Sp.	0.3	0	1.3	0	0	2	
Mean height of vegetat.	36.6	40	53.3	51.6	58.3	35	10.5 ns

Table 4.5 contd

	HAW WOOD	PEGWELL WOOD	TOCKINGTON PARK	KINGTON GROVE	KNAPP WOOD	WETMOOR WOOD	X^2
Classi-fication							
Peterken	2Aa Wet Ash/ Maple	9Ab Pedunculate Oak/Hornbeam	1Ba Oak/Wych Elm	3Aa-3Ab Peduncalute Ash/Hazel/ Ash	1Ba-1D Ash/Wych elm	2Aa Wet Ash/ Maple	
Bunce	6 Twayblade/ Ivy	1 Nettle/ Bramble	7 Wood Sedge/ Bramble	9 Bluebell/ Bramble	5 Ground Ivy/ Dog's Mercury	7 Wood Sedge/ Bramble	

* Species may occur in more than one layer, therefore numbers may not add up.

† Percentage converted to m^2 covered to enable chi squared to be calculated.

maple variety, elements of 9Aa, the birch hazel variation could also be seen.

Having completed the classification based on Peterken´s method, the results appeared rather unsatisfactory. Only two of the sites, Haw and Tockington Park could be seen to fit easily into their allotted categories. In other situations the ground vegetation and the tree species were not compatible and the presence of Quercus petraea also caused problems. Because of these problems, the sites were reclassified using the Merlewood method. Although the species list at each site was based on a larger area than that used in the Merlewood scheme, this was borne in mind when using the key. Very rarely was a step determined by a single species and in all cases where it was, (and most where two species separated the alternatives) the species concerned were deemed to be wide spread and representative of the woodland.

For most of the sites, the Merlewood classification described the woods fairly well. Wetmoor was linked this time to Tockington Park as type 7, woodsedge and bramble. This plot type also had ash and oak trees with hazel and field maple. All the other sites emerged as different plot types. Knapp wood was type 5, ground ivy and dogs mercury. With a dominant species of bramble and tree species including ash and oak with hazel and field maple, this description fitted well. Bluebell and bramble, type 9 described Kington Grove very aptly. With the species list of oak, sycamore, ash and hazel, presence of bracken and even the comment of high litter depth (see Figure 4.4) Kington Grove could almost be a ´type´ wood.

The two sites nearest to the smelter did not fit into their appropriate plot types quite so well. Pegwell wood keyed out as plot 1, nettle and bramble. Ash, beech and sycamore were all found at this site, as were goosegrass, elder and ivy. However perhaps the most obvious features of this site were the hornbeam trees and ground cover of herb robert, neither of which occur in the description of plot type 1.

Haw emerged as plot 6, twayblade, ivy type. Despite the unpromising name (twayblades being absent from all the sites) many features of the type did fit those of Haw. Trees and shrubs include ash, oak, field maple and hazel. Bramble and enchanters nightshade were two of the listed plot dominants.

It is interesting to note that the south west region of Britain provided over 30% of the examples for all these plot types, except for plot type 9, examples of which are scattered all over the country.

Neither of these classification methods placed all six woods in the same category, which is what might have been hoped for. On a purely subjective basis, Pegwell wood and Wetmoor seem the most different from the other four, however the classifications do not confirm this. The Peterken stand system linked Wetmoor with Haw and Tockington Park to Knapp wood. In contrast the Merlewood plot type linked Wetmoor with Tockington Park. Differential degree of disturbance is a problem, which probably also affects the classification systems.

In conclusion although all six sites may have derived from the same original type of woodland, they clearly have different features now. Obviously, for the purposes of this investigation it would have been better if they were all classified as one plot or stand type but, in the area available the chances of finding six such woodlands is slim and the effect of pollution ie. distance and direction from the smelter was an important factor in determining the choice of sites used.

4.4 MOSS BAGS.

4.4a Introduction.

Chemical analysis of the leaf litter and soil can reveal the metal concentrations available to the decomposers in their food source. However, the levels of contamination have built up in these sites over many years. Coughtrey *et al*. (1979) considered that the accumulation of leaf litter at contaminated sites was the result of build up from over 20 years and due to progressive build up of heavy metal concentrations after the opening of the smelter in 1929. Thus it was considered important to establish the current deposition of pollutants at each site used, as an adjustment to and for comparison with levels in the leaf litter.

Various types of equipment are available in order to measure air concentrations and deposition at ground level. Examples are, the dustfall canister and the British standard deposit gauge, both of which collect into water (for further details see Perry & Young 1977). These are generally expensive and not feasible for a study which involved sampling at several sites and where public access gave a possible vandalism problem. Annual vegetation or leaves from deciduous trees can give indications of relative metal levels at sites (Little & Martin 1972) but, as these organisms are rooted into the soil, uptake through the roots may occur as well as deposition on the leaves. Martinez _et al_. (1971) used the Bromiliad, _Tillandsia usneoides_ to demonstrate elevated lead levels close to busy roads. As it is an epiphyte, this plant is not rooted into the ground. However, Bromiliads are not found in northern Europe, consequently European workers have used Bryophytes instead. Barclay-Estrup & Rinde (1978) sampled _Pleurozium schreberi_ and _Hylocomium spendes_ in urban and rural sites and showed differences in lead and zinc levels between the sites but not between the moss species. Ruhling & Tyler (1968) used the two previous species and _Hypnum cupressiforme_ and sampled all over

Sweden. They were able to determine a gradient of lead levels between the industrialised and the rural areas. Herbarium specimens were also analysed in order to look at the variation of lead levels over time. Yule & Lloyd (1984) used *Hypnum cupressiforme* to monitor levels of several metals in Scotland while Tyler (1972) looked at levels in Scandinavian mosses. Burton (1986) presents an extensive review of the use of indigenous mosses for monitoring levels of metals. The use of mosses in this way can indicate levels of metals present at a particular site, but does not show amounts deposited. Goodman & Roberts (1971) were the first to transplant the moss *Hypnum cupressiforme* growing on logs in clean areas to polluted regions. Although plants transported to the most polluted site died after 6 weeks, concentrations in the moss increased. Goodman & Roberts (1971) then continued by removing moss from clean sites and hanging it in nylon bags in polluted sites. These original moss bags contained *H. cupressiforme* but Martin & Coughtrey (1982) present a table comparing different species and show that species of *Sphagnum* are the best. *Sphagnum* has a high ion exchange capacity (Clymo 1963) and can also hold large amounts of water so that the majority of rain water falling on the bags is retained. *Sphagnum* moss bags have now been used extensively (Gill *et al*. 1975, Little & Martin 1974, Coughtrey 1978). A full description of the method is given in Martin & Coughtrey (1982) where the simplicity and inexpense of the method is also recorded. Coughtrey (1978) has shown the importance of positioning the bags and also demonstrated that the metal input (Cd) to the moss bags could be almost wholly accounted for by the proportion of winds blowing in each direction over the exposure time. Whilst noting that wind direction and smelter activity are important factors influencing deposition of the metals, the intention of using moss bags in the present study is to give a general impression of current deposition rates at each site. The topic of the biological monitoring of heavy metals is reviewed by Martin & Coughtrey (1982).

4.4b. Methods.

Small nylon hair nets (as used by Martin & Coughtrey 1981) were filled with approximately 600mg of dry *Sphagnum* moss and then moistened with deionised water. 5 such moss bags were hung at each site and 5 retained as controls. At the sites, the bags were attached to shrubs and trees with rubber bands at a height of 2m. The positions were close to the trapping grid in the centre of the woods. The bags were left in place for four weeks at a time and when removed were replaced by fresh ones in identical positions. The exposure time was chosen to coincide with trap collections and was also similar to that used by other workers (Coughtrey 1978, Little & Martin 1974). Over a period of a year only 10 bags were not recovered and a couple were reduced in size, probably due to interference by birds. When returned to the laboratory the moss bags were dried, weighed and analysed for the four metals, lead, cadmium, copper and zinc.

4.4c. Results and discussion.

The results obtained are very variable and on many occasions the values for the control bags exceed those from the sites. Appendix 5 gives the mean value for each site (for the four metals) from which has been subtracted the mean value of all the controls for the whole year. Results expressed as deposition per day (Coughtrey 1978, Muskett 1976) would not help in the understanding of these data and so were not calculated. The results are summarised in Table 4.6 as ratios of means for the year, expressing the lowest value as 1. Thus giving relative pollution rates for the six sites. For comparison, data for the leaf litter, F/H layers and soil are given, expressed in the same form.

The deposition at Haw is greatest for all the metals analysed, and, with the exception of copper, the ratio for the moss bags is much lower than that for the various soil layers. This is to be expected, as the values in the moss bags at the polluted site are due only to an accumulation over 14 days, as opposed to 14 or more years in the leaf litter. The clean sites, cannot in comparison, be cleaner when observed over a shorter time span than for a longer one. Pegwell wood, for the majority of metals has the next greatest deposition of metals, but the relative ordering of the following four sites is not consistent. Unfortunately,

Table 4.6

RATIOS OF METAL CONCENTRATIONS IN MOSS BAGS AND LEAF LITTER FOR EACH SITE

Obtained by dividing by the lowest value in each category.

	WM	KW	KG	TP	PW	HW
CADMIUM						
Moss	1.439	1	1.582	1.039	2.233	16.796
Litter	1	1.668	3.386	2.777	5.456	32.893
F/H	1	1.462	1.509	1.951	6.478	96.19
Soil	1.170	1	1.474	1.169	4.618	49.302
COPPER						
Moss	1.818	1	1.258	1.289	1.774	11.805
Litter	1	1.116	1.482	1.439	1.953	12.352
F/H	1	1.048	1.393	1.598	2.479	10.056
Soil	1	1.07	1.059	1.165	2.215	3.156
LEAD						
Moss	1.12	1.04	1.51	1	1.86	2.62
Litter	1	1.522	2.983	3.827	4.20	34.789
F/H	1.069	1	2.811	4.873	3.309	35.291
Soil	1.107	1	2.638	5.561	1.936	6.601
ZINC						
Moss	1	1.18	1.734	1.33	2.165	9.822
Litter	1	1.515	2.138	2.602	3.452	18.666
F/H	1	1.193	1.247	3.204	3.868	26.584
Soil	1	1.044	1.167	3.509	3.246	15.324

as the data are rather variable, it is not possible to examine variation in deposition over the year. Comparison of the deposition with predominant wind directions is also not worthwhile.

In conclusion, the deposition on to the moss bags has shown that Haw wood is continuing to receive the highest levels of pollutants from Avonmouth of the six sites studied. Pegwell wood has the next highest levels of deposition, but the other sites can be seen as more similar and not separable on general terms.

Burton (1986) lists many studies using Sphagnum moss bags and reports no particular problems with the method. Because the unexposed moss bags used in this study contained measurable amounts of metals this has obscured any deposition patterns at sites where the deposition rates were low. With hindsight it would have been desirable to wash the moss in an acid rinse before exposing it at the sites, particularly those with low deposition rates. This was undertaken by Muskett (1976) who rinsed the moss in dilute nitric acid for 3 days before using it in bags, he then had no problem measuring low levels of deposition in rural areas. A longer exposure time may also have improved the situation. There is no obvious reason why the control moss bags contained elevated levels of metals, but unfortunately because they did, the data were not as useful as they might have been.

Chapter 5.

EFFECTS OF HEAVY METAL POLLUTION ON SELECTED DECOMPOSERS.

5.1 Worms.

5.1a Introduction.

The importance of earthworms in helping to break down organic matter in the soil has been realised since Darwin's time (Darwin 1888). Attention is often focused on the British species *Lumbricus terrestris* which pulls leaves down into its burrow, the archetypal mixer and aerator of the soil. The biomass of worms can reach staggering proportions, 0.75-1.0 tons of fresh weight per acre being estimated by Raw (1962) for a British apple orchard. This population was clearing 0.5 tons dry weight of leaves per acre in one winter. In areas devoid of worms, for example orchards with a long history of insecticide and fungicide spraying, not only is there a build up of organic matter on the surface (Raw & Lofty 1959, Satchell 1967) but the soil profile is changed. The crumb structure becomes poor (Raw 1962, Hirst *et al*. 1961) and the soil becomes sub-angular and blocky (van de Westeringh 1972). Subsequent addition of worms improves the soil (Van Rhee 1977) although the worms may not survive for long. Satchell (1967) considers that,

"conditioning plant remains for microbial decomposition seems to be the most important action of Lumbricidae in the ecosystem."

From this it is intuitive that the absence of worms can cause a stock pile of undecomposed organic matter. Both essential and nonessential elements will be locked up and the ecosystem will be disrupted.

Heavy metals have been shown to have deleterious effects on worms, for example Jaggy & Streit (1982), Wade et al. (1982) and Ma (1984). Other studies have examined the interaction between worms and heavy metals in a variety of different situations. Sewage sludge, with its associated high concentrations of heavy metals, and its application to the land has been well documented. Studies have examined the effect of worms on the sludge, (Hartenstein & Hartenstein 1981) and the effect of sludge on the worms (Hartenstein et al. 1981) and the implications for higher links in the food chain (Beyer et al. 1982). The reaction of worms to insecticides (Polivka 1951) and fungicides (Raw & Lofty 1959) has been monitored. Road side populations have been sampled for lead and other metals (Gish & Christensen 1973 and Czarnowksa & Jopkiewicz 1978) and mine spoil sites sampled likewise (Ireland 1975a, 1977a). Worms in the proximity of industrial sites have been studied, Bengtsson et al. (1983a) recording populations and metal concentrations in animals near a brass mill, and Ma et al. (1983) and Beyer et al. (1985) in those close to zinc smelters. The situation at Avonmouth has been recorded in pastureland by Wright & Stringer (1980) and in woodlands by

Martin & Coughtrey (1976) and Hopkin *et al*. (1985b). The aim of the present study was to look at worm populations in the woodland sites used for sampling other animal groups and to determine the levels of metals in those animals present.

5.1b *Methods of collection*.

Satchell (1971) lists three main methods of capturing worms: sorting through soil samples by hand, trapping and expelling from the ground. Although laborious and probably inefficient, hand sorting is still being used as a collecting method (Helmke *et al*. 1979, Ma *et al*. 1983). A method of sorting more efficiently was described by Raw (1960) where vegetable matter was teased apart over a bowl of water containing a small amount of formalin to make the worms wriggle. The rest of the material was sieved and immersed in magnesium sulphate solution so that the worms float to the surface. Although more juvenile worms were recovered this way than simply sorting by hand, it is obviously more time consuming.

In low density areas Satchell (1971) listed baiting and trapping as useful in comparative studies. Dung is a good bait for many species and Satchell (1971) described a trap consisting of an earthenware pot. Sims & Gerard (1985) also mention pitfall traps. For animals to be caught by these methods, they must be active and on the surface of the litter. In contrast, methods of collection which involve expelling worms from the soil will sample individuals that may be deep down.

Using an electric current animals can be extracted up to 3 feet from the electrode (the depth depends upon the length of the electrode). The disadvantages of this method are that animals within 3 inches of the electrode are killed, and satisfactory extraction requires a current of 3 amps for 40 minutes (Satchell 1955a), thus specific equipment is required. More usual methods of expulsion are those involving chemicals. Whilst Sims & Gerard (1985) listed mustard and water, more commonly potassium permanganate or formalin is used. Evans & Guild (1947) recommended the use of 0.25 oz potassium permanganate in 1 gallon of water for an area of 2.7 x 2.7 feet. They considered the rate of recovery of the worms to be high; however Svendsen (1955), whilst achieving the same estimate of numbers as Evans & Guild (1947) when using potassium permanganate, found this was low in comparison to hand sorted samples. Satchell (1967) considered potassium permanganate not to penetrate the soil well and to produce a variable response from the worms. Another disadvantage is that the worms disintegrate immediately. The use of formalin was first proposed by Raw (1959) and since then has become widely used in ecological studies (e.g. Bengtsson et al. 1983a, Andersen 1979). Raw & Lofty (1959) found dilute formalin more effective than potassium permanganate which underestimated the population. In a direct comparison Raw (1959) estimated the population of *Lumbricus terrestris* in an orchard using various methods. The formalin figure of 20.5 per m^2 compared favourably with counting the burrows by eye (18.5). The potassium permanganate estimate was 5.2 and hand sorting 3.1. This last figure is probably low because of the inadequate depth reached. Indeed, Satchell (1969) recorded that formalin is a good method for *L. terrestris* which has deep vertical burrows, however it was less efficient for horizontal burrowers. The temperature and moisture of the soil have an effect on the extraction efficiency and Satchell (1969) gives a formula for correction for *L. terrestris*. Nordstrom & Rundgren (1972) showed that the addition of detergent to the formalin does not increase the efficiency, and a comparison of different species in this study indicates that hand sorting is more efficient only for *Allolobophora* species because any animals in facultative or obligative diapause do not respond. This factor was used by Nordstrom (1975) to estimate the percentage of active worms at different times of the year. Satchell (1969) stated that the usual procedure at the Institute of Terrestrial Ecology, Merlewood, for worm extractions is to apply 2 gallons of 0.165% formalin to quadrats $0.5m^2$. This is repeated twice with 10 minutes between applications, ie. a total of 6 gallons added. Up to 7 applications were experimented with by Satchell (1971) but 95% of the worms were removed after 3 doses of formalin. The Merlewood method is now the most widely used.

5.1c Methods of capture used in this study.

Whilst some worms were captured in the pitfall traps intended for ground running arthropods, these were mostly litter dwelling species. The numbers caught in this way are discussed below. However another more specific method was required to gain an estimate of deeper living animals at each site. Formalin was chosen as the most appropriate extractor in the circumstances, the main disadvantage being the volumes of water needed to be carried to the sites. In general the Merlewood method was followed, although the quadrats were smaller, 50 x 50cm, so the volume of liquid was reduced likewise. Hence, 25ml of 4% formalin was added to 5l of water; in each of 5 randomly placed quadrats 2.5l of this solution was slowly poured; at 10 minute intervals this was repeated until each quadrat had received 7.5l. Earthworms coming to the surface at any stage were thoroughly washed in water to remove any traces of formalin and placed in plastic bags together with a small amount of leaf litter for the journey. 10 minutes after the last application of formalin to the quadrats, they were dug into to a depth of a few centimeters to assess the penetration of the liquid.

Unfortunately, the collections were made on 15th, 16th and 18th October 1986, just at the end of a period of hot dry weather. The formalin solution did not penetrate as well as was to be expected and, in particular at Kington Grove the movement of liquid in a lateral fashion was a problem.

Worms were removed from the plastic bags in the laboratory and transferred to sandwich boxes containing washed china clay waste gravel. The boxes were kept in a cold room for 36 hours. The gut contents of the worms account for a large proportion of the metals found on analysis if not cleared out. This is not important if the passage of metals up the food chain is to be assessed (Stafford & McGrath 1986), because predators ingest the worm and its gut contents. In the present study, levels of metals in the worms themselves was of interest.

Van Hook (1974) starved animals kept on damp filter paper for 4 days to achieve emptying of the gut. Other workers have starved animals and then followed this with dissection of the gut (Andersen 1979, Beyer _et al_. 1985). However Ash & Lee (1980) advise against this because the main site of accumulation of many of the metals is close to the intestine. Stafford & McGrath (1986) consider starving the animals to be time consuming and describe a method of using acid insoluble residues to establish levels of metals in the gut contents.

The use of china clay gravel in the present study was considered a better medium than filter paper to starve the worms on. The worms could move amongst the stones to expel the current gut contents, but the particle size was too large for ingestion. After 36 hours no gut contents could be seen in the worms except for three small specimens which died and were not used for the metal analysis. Worms were identified using Sims & Gerard (1985) and Edwards & Lofty (1977). Names follow Sims & Gerard (1985). Animals were dried, weighed and analysed for the four metals.

5.1d Results and discussion.

5.1d i. Pitfall trapping data.

The list of animals caught whilst pitfall trapping are given in Table 5.1. The polluted site, Haw, caught the largest number of species and the second highest number of individuals. It does not therefore seem to be lacking in numbers of worms. This is in contrast to what may be expected since Wade *et al*. (1982) estimated worms to be 25 times more abundant in control plots than in those treated with sewage sludge containing high concentrations of metals. Andersen (1979) found an increasing dominance of two species in sludge treated fields.

The method of capture employed here is heavily biased against those animals living deep in the soil, for example *Octolasium cyaneum* and adult *Allolobophora chlorotica* and *Apporectea caliginosa* (Edwards & Lofty 1977). Surface or near surface inhabiters like *Lumbricus castaneus* and *L. rubellus* are better represented in the traps. Haw has a

Table 5.1

NUMBERS OF WORMS CAUGHT WHILST PITFALL TRAPPING

	HAW WOOD	PEGWELL WOOD	TOCKING-TON PARK	KINGTON GROVE	KNAPP WOOD	WETMOOR WOOD	X^2
Allolobophora chlorotica	1						
Aporrectodea caliginosa	1	1			1	1	
Aporrectodea rosea	15	6	1	1		4	
Dendrobaena octaedra		4					
Dendrobaena rubidus					1		
Eisenia fetida					1		
Eiseniella tetraedra						2	
Lumbricus castaneus	17	12	17	9	7	7	
Lumbricus rubellus	7	11	3	5		2	
Lumbricus terrestris	2						
Octolasion cyaneum	1						
Immature Worms		15	3	5		5	
No. of Species	7	5	3	3	4	5	2.56 $p>0.05$
No. of Individuals	45	49	24	20	10	21	41.9 $p<0.01$

greater depth of leaf litter which may be a contributing factor to the high numbers in the traps at this site.

Satchell (1955b) classified worm species into three groups depending on their acidity preferences. These being, acid tolerant, acid intolerant and ubiquitous species. Piearce (1972) has since examined a wide range of sites to explore this idea more fully. Both *L. castaneus* and *L. rubellus* found throughout the sites are considered ubiquitous species, as are many litter feeders. The sites sampled are mostly rather acidic, which may also explain the low numbers of the *Allolobophora* and *Apporectea* species which are mostly acid intolerant.

5.1d ii. *Formalin extracted animals - species and numbers.*

The animals captured by means of formalin extraction are listed in Table 5.2. Also shown are the total number of species, number of individuals, number of animals per m^2 and biomass per m^2 as dry weight. Biomass is potentially more useful than simply numbers of animals because of the larger amounts of material ingested by the larger sized individuals. Thus biomass gives an indication of the effectiveness of a population in breaking up organic matter. Although Abrahamsen (1973) argues that body surface area may be the best method of describing the activity of worm populations because of the higher metabolism in smaller

Table 5.2

NUMBERS OF WORMS CAUGHT FROM EACH SITE BY FORMALIN EXTRACTION

(Totals of 5 x (0.25 x 0.25m) quadrats)

SPECIES	HAW WOOD	PEGWELL WOOD	TOCKING-TON PARK	KINGTON GROVE	KNAPP WOOD	WETMOOR WOOD	X^2
Allolobophora longa					2		
Aporrectodea rosea					1		
Octolasion cyaneum	5						
Lumbricus terrestris			3		2	3	
Lumbricus rubellus		17	2	1	3	2	
Imm. *Lumbricus* Sp.	2	3		1	3	4	
No. of Species	2	2	3	1	4	2	2.29 $p>0.05$ ns
No. of Individuals	7	20	5	2	11	9	
No. of Individuals/m^2	5.6	16	4	1.6	8.8	7.2	17.24 $p<0.01$ **
Biomass (g dry wt/m^2)	0.28	1.02	1.09	0.073	0.718	1.176	1.45 $p>0.05$

animals, biomass was considered the most appropriate in the present study.

As seen in Table 5.2 the number of individuals per m^2 is significantly different between the sites, but numbers of individuals and dry weight biomass are not. Again Haw wood does not have the lowest number of animals nor the lowest biomass. Wright & Stringer (1980) found no evidence of a reduced population of worms in pasture close to the Avonmouth smelter but they were comparing two different habitats as well. These results are in contrast to Bengtsson et al. (1983a) who recorded density and biomass to be inversely proportional to distance from the brass mill. Sampling in the Swedish study commenced only 175m from the mill, which is much closer than in the present study, and their control site had what is probably an exceptionally high number of worms. Satchell (1983) records that the numbers of worms in oak forests may vary from 0 to 192 per meter squared, so a large amount of variation is to be expected. A previous study of woodland near Avonmouth (Hopkin et al. 1985b) revealed a large difference in numbers of species, individuals and biomass between Wetmoor and Hallen, a similar wood close to Haw but more heavily contaminated with heavy metals. The results are shown in Table 5.3 for comparison with the present study. The sampling methods used were very similar, although the previous study was undertaken later in the year. Both the numbers per m^2 and the biomass for the two sites are

Table 5.3

NUMBERS OF WORMS PER M^2 COLLECTED FROM HALLEN AND WETMOOR IN 1982

(From Hopkin *et. al*. 1985 and Mould 1983)

	Hallen	Wetmoor
Lumbricus rubellus	17	3
Lumbricus terrestris		29
Allolobophora longa		4
Allolobophora calaginosa		30
Octolasion cyaneum		9
No of species/m^2	1	5
No of Individuals/m^2	17	75
Biomass (g dry wt/m^2)	1.61	15.93

considerably higher in 1982 than in 1986. This may be due to the difference in time of the year of sampling, but is more likely to be caused by the very dry spell prior to extraction in 1986. One aestivating Allolobophora was discovered at Knapp wood and it is probable that other worms were not extracted as they were in an inactive state. The poor penetration of the formalin solution is likely to account for the limited extraction of some of the deeper living species and this problem may also account for the low numbers found at Kington Grove. The deeper leaf litter at Haw may result in less worms aestivating, thus more were available for capture.

Although the worm sampling at the sites did not yield as many animals as expected, even from the unpolluted woods, the data from those captured has proved interesting. Haw, the most polluted site does not have the lowest number of individuals, nor the lowest biomass of worms of the sites studied. From the pitfall trapping data it seems that perhaps there is a higher proportion of surface active, litter inhabiting worms. With the increased depth of litter at this site, there is abundant food so that competition for food, considered an important factor in limiting the populations of worms (Satchell 1967) cannot be a problem. More sampling is obviously needed at these sites and others in order to elucidate the problem further.

5.1d iii. Metal concentrations in the worms.

The metal concentrations found in the worms are summarised in Table 5.4 for all species grouped together. Analysis of variance for dry weight and for all metals were significant, showing differences between the sites. Animals from Haw wood had the highest concentrations for all four metals, and on the whole the further from the smelter the lower the concentrations. An exception to this is the high lead content at Wetmoor.

Martin & Coughtrey (1982) show a table giving the highest recorded metal levels in earthworms. In accordance with this, the copper values found at Haw are well below the 46ppm maximum. The highest lead concentrations recorded of 7593ppm, although also at a smelter site, is considerably larger than those recorded here. Roadsides yielded concentrations of 1950ppm zinc which is a little in excess of those in the present study. The cadmium recorded in worms from Haw (235.6 ppm) greatly exceeds that of 144ppm previously the highest concentration (from another study in Avonmouth). Although larger by more than half as much again, the Haw value is realistic. Hopkinson (1986) recorded 190.8 in *L. rubellus* from a nearby wood, and Morgan (1987), 577ppm in the same species from mining sites.

Table 5.4

MEAN METAL CONCENTRATIONS (μg/g) FOR ALL WORMS AT EACH SITE

SITE	N	DRY Wt. (g)	CADMIUM	COPPER	LEAD	ZINC
H.W.	7	0.05 ± 0.014	235.6 ± 25.8	11.78 ± 3.32	433 ± 101	1345 ± 243
P.W.	19	0.064 ± 0.006	141.3 ± 9.56	7.8 ± 0.84	14.28 ± 2.64	1471 ± 147
T.P.	4	0.317 ± 0.171	45.9 ± 1.7	5.75 ± 1.8	24.8 ± 17.8	562 ± 110
K.W.	11	0.08 ± 0.023	5.51 ± 1.67	5.69 ± 1.6	1.99 ± 0.57	643 ± 1.4
W.M.	7	0.202 ± 0.117	31.45 ± 5.56	3.47 ± 1.2	74.0 ± 42.1	430.8 ± 72.8
F		3.27	51.93	2.81	21.85	7.45
p		<0.05 *	<0.01 **	<0.05 *	<0.01 **	<0.01 **

In general the levels of metals in worms have been shown to be correlated with those in the soil (Beyer et al. 1982, Helmke et al. 1979, Martin & Coughtrey 1982). There is much discrepancy between different authors as to which metals are accumulated and which are not. Table 5.5 illustrates this discrepancy. Only cadmium is consistent, with all workers agreeing that it is accumulated in worm tissue. There are several reasons for these differences of opinion. The first is differences in methodology and analytical techniques. The second is a difference of understanding of the term accumulation, Hartenstein et al. (1980) have discussed this problem in more detail. Accumulation can be taken to mean either accumulation above that of the food source or concentrations of metals in the tissues exceeding those of animals in unpolluted environments. The first meaning can be described in more useful terms by the concentration factor, or the ratio of metal concentrations in the animal compared to its food. Concentration factors for various studies including the present one are given in Table 5.6.

It has been reported before (Beyer et al. 1982, Ireland 1983) that concentration factors are higher in uncontaminated soils than contaminated ones. This is shown in the present study for copper, lead and zinc but not cadmium. In general, for all studies cadmium appears to be accumulated in worm tissues to a greater extent than the surrounding medium. For the other metals, most studies

Table 5.5

ARE METALS ACCUMULATED OR REGULATED IN EARTHWORMS?

AUTHOR	METAL			
	Cd	Cu	Pb	Zn
Ireland (1979)		Regulated	Accumulated	Regulated
Beyer et.al. (1982)	Accumulated	Not accumul.	Not accumul.	Accumulated
Van Hook (1974)	Accumulated		Not accumul.	Accumulated
Hartenstein et al. (1980)	Accumulated		Accumulated	Not accumul.
Helmke et al. (1979)	Accumulated			Accumulated
Roberts & Johnson (1978)			Accumulated	Accumulated
Czarnowska & Jopkiewicz (1978)	Accumulated	Accumulated	Accumulated	Accumulated
Ma et.al. (1983)	Accumulated		Accumulated	Accumulated
Ash & Lee (1980)	Accumulated	Accumulated	Accumulated	

Table 5.6

CONCENTRATION FACTORS RECORDED BETWEEN WORMS AND THE SOIL IN WHICH THEY LIVE

AUTHOR	SITE	CADMIUM	COPPER	LEAD	ZINC
Gish & Christensen (1979)	Roadside	11.2		0.95	5.7
Ireland (1979)	Mine	0.7 - 1.7	0.01	0.1	0.2 - 0.7
Andersen (1979)	Sewage	23 - 39		0.24	
Beyer *et. al.* (1982)	Smelter	Lower ratios in contaminated soil than uncontaminated			
Wright & Stringer (1980)	Smelter	5.6 - 14.7		0.3	2.3 - 5.2
Van Hook (1974)	Smelter	17		0.2	8
Hartenstein *et. al* (1980)	Sewage	3.9		0.04	0.06
Czarnowska & Jopkiewicz (1978)	Roadside	17.1 - 27.3	0.56 - 0.8	0.36 - 0.47	7.3 - 17.5
Present study - Haw	Smelter	3.05 - 11.87	0.077	0.18 - 0.38	0.44 - 0.97
Wetmoor	Smelter	0.25	2.06	1.04	3.78

indicate that although levels of metal in the tissues may rise in concordance with those in the soil, concentrations in the worms on the whole remain the lower of the two. The metal values given for soil and leaf litter are total concentrations not that available to the worms. This will influence the concentration factors (Ma 1982).

Many factors may affect the uptake of metals and their concentration in the tissues. Differences between species of worm have been noticed. For example Ash & Lee (1980) found variation in uptake of lead between *A.chlorotica* & *L. terrestris* and Andersen (1979) showed differences in the behaviour of cadmium in various *Allolobophora* and *Apporectea* species. In the present study, differences were observed between *Lumbricus* species and *Octolasium cyaneum* at Haw as illustrated in Table 5.7. Although only zinc is significantly different, the standard errors are large due to the low sample number.

Differences between mature and immature worms of the same species have also been recorded. Bengtsson *et al*. (1983a) found higher levels in the juveniles of *A. caliginosa* where as Ma (1982) found that adults had higher levels of cadmium, zinc and lead than the juveniles. At Pegwell wood, juvenile and adult *L. rubellus* were caught. Analysis of these (Table 5.8) shows that there is no difference between the concentrations of cadmium and zinc, but that copper is

Table 5.7

CONCENTRATIONS OF METALS IN THE TWO SPECIES OF WORM FOUND AT HAW

	Lumbricus sp.	*Octolasion cyaneum*
Cadmium	294.8 ± 27.1	191.2 ± 20
Copper	5.49 ± 4.92	16.5 ± 3.0
Lead	664 ± 144	260.1 ± 45.1
Zinc	679 ± 115	1844.6 ± 74

Table 5.8

METAL CONCENTRATIONS ($\mu g/g$) IN JUVENILE AND ADULT L. RUBELLUS FROM PEGWELL WOOD

	Immature	Mature	t	df	p
Dry weight(g)	0.05 ± 0.007	0.079 ± 0.007	2.85	17	0.012 *
Cadmium	154.2 ± 16.5	126.97 ± 6.6	-1.53	11.8	0.15 ns
Copper	5.51 ± 0.8	10.36 ± 0.99	3.81	15.9	0.0017 **
Lead	21.0 ± 3.86	6.82 ± 1.07	-3.54	10.4	0.005 **
Zinc	1563 ± 267	1369 ± 106	-0.68	11.7	0.51 ns

significantly higher in the adults and lead is significantly higher in the juveniles.

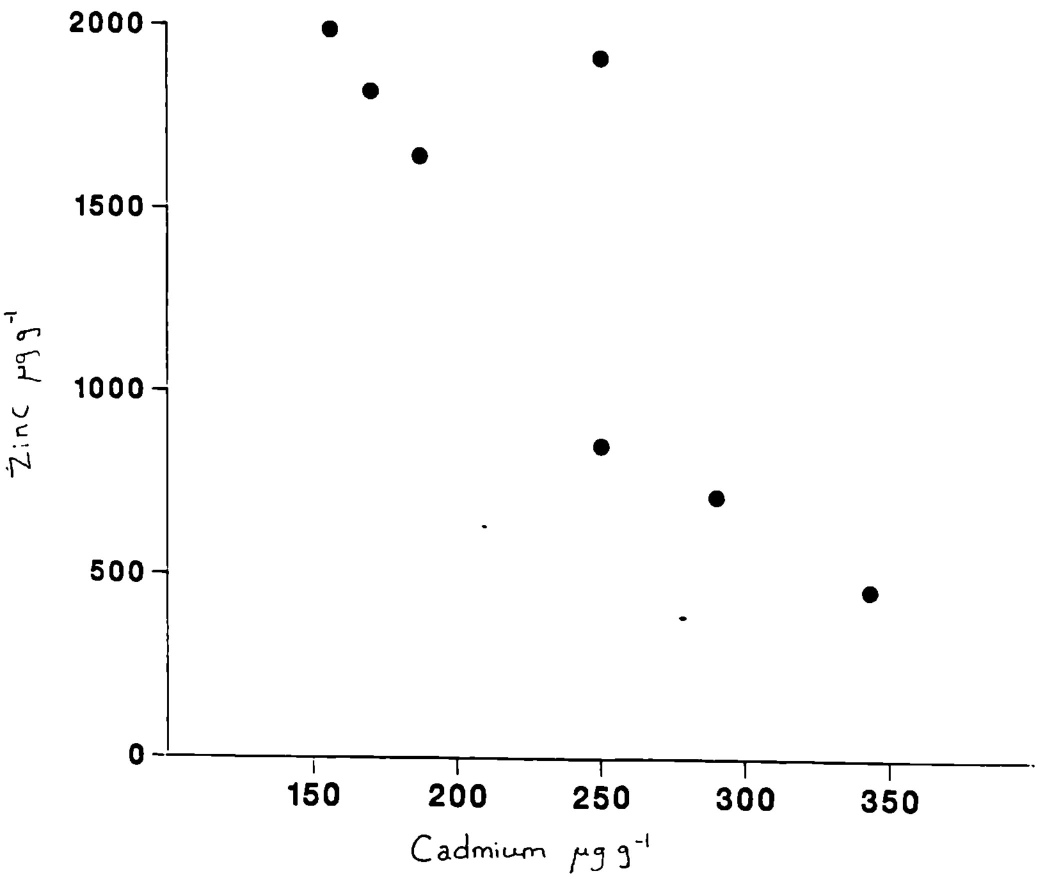
Ma (1982) reported that organic matter in the soil may play a significant role in the uptake of metals by worms. This was examined in more detail using L. rubellus (Ma et al. 1983) and it was concluded that organic matter was important in the uptake of only lead, this metal being accumulated more by worms in soils with a low organic content. This is in contrast to Gish & Christensen (1973) who found a positive correlation with metal in soils (and hence in worms) and organic matter. The latter finding is more to be expected as high metal pollution is believed to cause a reduction in number of decomposers, leading to a build up of leaf litter and organic matter. Any worms present may be expected to have high concentrations of metals themselves. Neither theory appears to be supported nor disproved by the present study, although organic matter content of the soils was not determined as such. Ma et al. (1983) also record an interaction with pH and it is possible that this is more important than the quantity of organic matter.

Satchell (1955b) records that pH of the soil is highly correlated with numbers of worms, and comments that extreme soil acidities may be important in limiting distributions. The pH may also have an effect on the uptake of metals by worms. Ma et al. (1983) reports L. rubellus to accumulate cadmium, zinc and lead more strongly in soils with a low pH, but copper was not affected. Beyer et al. (1982) also recorded a decrease in the cadmium concentration in worm after liming the soil, which increased the pH. In contrast Gish & Christiansen (1973) found pH to have no effect on the metals they recorded, which excluded copper, although the toxic effects of copper were apparently reduced by Ma (1984) when the pH of the soil was altered from 4.8 to 7.1.

Interactions between the uptake of metals have also been proposed. Ireland (1979) considered that soil copper may influence the metals accumulated and Petering (1974) notes that lead absorption is reduced when there are high levels of copper in the diet. There is no evidence for this in the present study, the highest level of dietary copper being at Haw which corresponds to the highest lead levels in the worms. It is interesting that *Octolasium cyaneum* shows higher copper and lower lead levels at Haw than *Lumbricus* species in the same habitat. This may be due to the selection of different food types, *Lumbricus* feeding mainly on organic fragments where as *Octolasium* takes in a higher proportion of mineral soil (Piearce 1978).

Beyer et al. (1982) found that high zinc in the soil reduced the concentration of cadmium in the worms. *Octolasium* at Haw shows higher zinc and lower cadmium than *Lumbricus* species. A plot of cadmium and zinc content in animals from Haw showed a strong relationship, however the data were very limited (Figure 5.1). The correlation between cadmium and

Figure 5.1 Plot of zinc concentration against cadmium concentration for worms found at Haw wood (site 6).


2000
1500
1000
500
0
Zinc µg g⁻¹
150
200
250
300
350
Cadmium µg g⁻¹

zinc content is significant and negative ($r=-0.855$, $df=6$, $p<0.05$).

The concentrations of metals found in the worms at Haw are high, particularly cadmium. Whilst metals are obviously toxic to the worms in high concentrations Jaggy & Streit (1982) found that most O. cyaneum were killed within a day when exposed to concentrations of 400ppm copper) there may be other sublethal effects which limit the potential population boom the abundant food supply might suggest ought to be occurring. For example Hartenstein et al. (1981) found that high levels of cadmium and copper inhibited the growth rate of Eisenia foetida, and Ma (1984) observed a decrease in cocoon production in L. rubellus exposed to high copper doses. These two studies indicate that life history parameters may be upset by the metals, fewer young will be produced and the growth rate to reproductive state slowed down. In addition, Bengtsson et al. (1983a) when analysing different anatomical parts of worms for metals found the highest concentrations in the seminal vesicles and the cerebral ganglion. They proposed that the former may upset reproductive capabilities and the latter affect burrowing activity, exposing the worms to high risk of desiccation and predation.

It seems possible that metal concentrations in the worms themselves may be altered by the action of the calciferous glands. Shedding of the posterior region of the body to remove excess nitrogenous waste (Sims & Gerard 1985) is not a method by which lead and zinc are lost (Ireland 1977a) thus the metal levels recorded at Haw are probably typical of most of the worms found there. Differences between species obviously do occur however it seems probable that the chief limits to the population growth of the worms at Haw are the constraints imposed on growth and reproduction caused by the levels of metals in the soil. The next stage in the investigation should be a study of the age structure of the worms and their reproductive capacities on polluted soils.

5.1e SUMMARY.

1. Worms were sampled by pitfall trapping and extraction by formalin. Metal concentrations were determined in those captured by the latter method.

2. Differences were found between the number of worms caught by pitfall traps at each site. The polluted sites did not have the lowest numbers. This indicates that the surface active worms were not reduced greatly.

3. The number of individuals caught using the formalin method varied significantly between the sites. The biomass (dry weight per unit area) did not. Again the polluted sites did not show substantially reduced numbers of animals.

4. Numbers of animals per m^2 were lower than the same (or similar) sites sampled in 1982. This is attributed to differences in sampling efficiency.

5. Concentrations of cadmium, copper, lead and zinc were greatest at Haw and differences between the sites were significant.

6. Differences in metal concentrations were found between species and between mature and immature forms of the same species. Levels of metals are not consistently higher in one species or one age class than in another.

7. The factors affecting uptake of metals into the tissues are discussed. From the limited data available a significant negative correlation was found between cadmium and zinc in mature worms from Haw.

8. It is concluded that food supply cannot be the limiting factor for worm populations at Haw. The abundance of organic matter should support a population which is higher than that found. The high concentration of cadmium, copper, zinc and lead may cause reduction in growth and reproductive capacity, maintaining the population at a lower than optimum level.

5.2 Microarthropods.

5.2a Introduction.

Both mites and collembola have been shown to be very active in the decomposition of leaf litter (Witkamp & Crossley 1966). Although some mites are themselves predatory, many of these micro-arthropods are valuable food sources for the larger predators, both specialists e.g. the Carabid *Loricera pilicornis* (Forsythe 1982) and the more generalist feeders e.g. the Carabid *Pterostichus madidus* (Davies 1953). The abundance and diversity of the mites and collembola in the polluted woods are therefore of interest.

Whilst pitfall traps capture the active soil/litter fauna, the method is not satisfactory for the less active and the smaller animals. Undoubtedly the most efficient method of removing all the animals from a sample of soil or litter is to sort through by hand. This method however has the serious drawback of being extremely time consuming and is also not very practical for the microarthropods. To overcome these problems several different methods have been devised following three main directions: sectioning, flotation and the Berlese-Tullgren type of extraction.

The techniques of sectioning generally used, follow the guide lines of Anderson & Healey (1970). Soil samples are embedded in gelatine and serial sections cut to enable identification of the arthropods present. Obviously identification can be a problem in such a situation, especially of juvenile animals. Pande & Berthet (1973) in a comparison of serial sectioning and Tullgren extraction, found that the former method took 60 to 70 times longer but was a better technique for studying vertical and horizontal movements and aggregations (of mites). Population studies were more accurate when Tullgren type methods were employed.

Flotation methods have been used by various workers e.g. Ladell (1936), Salt & Hollick (1944) and Salt et al. (1948). A general description of the process involved is given in Southwood (1980). Adaptations have taken place, for example Glasgow (1939) expanded the apparatus to hold larger samples and Belfield (1956) used a field version which required no special supplies such as electricity or running water. One of the biggest problems with this method is the separation of the arthropods from organic matter which also tends to float. Block (1966) pointed out that flotation was not a practical method when there is a high content of organic matter in the soil. The standard method of separating the floating items is that used by Salt et al. (1948) of mixing with benzene. Macfadyen (1953) however dismisses this method as ´unpleasant´. Hale (1964) achieved the same result by boiling the water containing the sample under reduced pressure, causing the plant material to sink. The efficiency of this method was recorded (Hale loc. cit.) as being 10 times as efficient as ordinary Tullgren extractors, there was no significant difference when compared with high gradient cylinders except for two species (of collembola). Macfadyen (1953) noted that flotation is good for large invertebrates but less satisfactory for small ones. It is also labour intensive taking 10 fold the amount of time as Tullgren funnels.

The Berlese-Tullgren method of extraction was recorded in 1971 by Edwards & Fletcher to be used by 74% of workers. Berlese in 1905 was the first to use heat to extract animals from soil and he was followed in 1918 by Tullgren who substituted a light bulb as the heat source. Variations in size and number of funnels have followed: perhaps the most important discovery since the innovation of the funnel technique was that of Haarlov (1947) who described the biggest source of error as being dew on the side of the funnel, in which animals become stuck. This can be avoided by keeping soil away from the side of the sieve. The gap is now often referred to as Haarlov's passage, and may be enhanced by the use of plastic tubing to make an air passage (Paris & Pitelka 1962).

Southwood (1980) summarised several types of Tullgren apparatus, several of which have been developed by Macfadyen (1961). Two basic types can be seen, the large funnel type, where heat is applied in some form at the top and the small canister type, where a steep gradient is formed in a shallow depth of substrate by heating at the top and cooling below. (Other forms have been used, such as the horizontal platform for extracting spiders devised by Duffey (1962) where heat is applied from one side.)

The heat necessary to drive animals downwards is generally increased as time proceeds. Temperature gradients between the top and bottom of the samples have been in the region of 60°C (Hale 1964) to 70°C (Block 1966). Haarlov (1947) indicated that with full heat, a temperature shock may paralyse the animals, so temperatures used should not be in excess of those of the maximum in the localities where samples are taken. Although some extractions only extend over a 24 hour period (Ford 1937), the majority have used regimes of three days (Block 1966) and longer (Paris & Pitelka 1962, Macfadyen 1952).

Humidity, like temperature, is also important in the different parts of the sample. Haarlov (1947) indicated that 90% relative humidity is optimal. Brady (1969) recorded humidity at different levels in the funnel throughout extraction. Whilst the top layer remained the driest from the onset of heat application, up until 8 hours, the bottom layer was drier than the middle. A complete gradient was only achieved after 13 hours. The catch of animals showed an initial peak, then, after 20 hours there was a flush of 50 - 70% of the total catch. Brady (*loc. cit.*) considered that this may be due to the switch in humidity, as until then there was no vertical gradient for the animals to respond to. One problem of sharp gradients was noted by Block (1966), who found that condensation occurring in the collecting canisters may have the effect of lowering the temperature gradient but will not effect the humidity gradient.

When using high gradient extractors an additional problem is involved, as it is necessary to use some type of killing fluid in the canisters which will not affect the progress of animals moving down through the soil. Although this is also important when using large Tullgrens, is is not so much of a problem due to the increased distance between the soil and the collecting vessel. In high gradient extractors the soil layer is thin, often 3-5cm (Block 1966, Pande & Berthet 1973, Paris & Pitelka 1962) and much closer to a relatively larger area of the killing fluid. For short extractions of up to three days, water has been used (Block 1966, Ford 1937), however mould does grow fairly quickly (Macfadyen 1961). Ethanol has been widely used (Pande & Berthet 1973) but Macfadyen (1961) considers it not suitable. Sunderland et al. (1976) found trisodium orthophosphate ($Na_3PO_4.12H_2O$) at a concentration of 80g/l to be ideal in their situation as it was safe and cheap. However it has the effect of colouring the animals pink which could at times be a disadvantage. Phenyl mercuric acetate was rejected by Macfadyen (1961) as it corrodes aluminium, the composition of the canisters used. Duffey (1962), however used this in conjunction with a fungicide, a bactericide and wettol, to reduce surface tension.

Macfadyen (1961) compared 1.5% potassium dicromate, 70% ethanol and water and found the first two substances not to be good. He also examined Nipagin (0.3% solution) and Agrimycin (0.1% solution) neither of which showed significant differences from water. As Nigagin did have fungal growth on it, Agrimycin was concluded to be the best.

One factor still in discussion, is the advantages of leaving a sample intact and simply inverting it, as opposed to breaking it apart, thus enabling animals to leave the soil through natural pathways. The intact method was proposed by Hammer (1944) and has been followed by workers such as Macfadyen (1955) and Block (1966). Pande & Berthet (1973) inverted the top 3cm of their samples and broke apart the lower 3cm. D´Hulster & Desender (1982), by contrast, sorted samples first, which as well as removing some of the obvious animals makes the sample crumbly and it dries quicker giving it a higher extraction efficiency. It seems that breaking up the sample is of benefit when considering large arthropods whereas leaving soil intact is advantageous for small animals.

Macfadyen (1953) concluded that whilst anti-condensation methods and high gradient techniques have improved the efficiency of extraction, it is unlikely that a single set of apparatus will prove 100% efficient for all groups.

5.2b Methods of extraction used.

5.2b i. General.

It was decided that, in the circumstances, Berlese-Tullgren apparatus was the best method of extraction to use for micro-arthropods. A set of apparatus was constructed in order to accommodate samples 16cm in diameter and 20cm deep. Three such samples were taken from each site every month for the duration of the pitfall trapping. Although this was undertaken, the results in terms of animals extracted were very disappointing, the majority being in the size range of those caught be pitfall traps, and all in rather low numbers. It was considered that strip lighting was not an effective source of heat and light and even in a cooled room a satisfactory gradient was never reached. Even on a scaled down model, the temperature differentials between the upper and lower surfaces of the core were not adequate without enclosing part of the apparatus. Condensation and fungal growth added to the problems. Eventually, it was decided to make use of two sets of apparatus in other localities (one large funnel type and the other a high gradient canister type) and to sample on only one occasion.

5.2b ii. Manchester Funnels.

The funnel room at Manchester University consists of 60 permanent Tullgren funnels in a room maintained at a constant 10^{o}C. The fibre-glass funnels are all situated with their large opening (30cm in diameter) flush with the bench top. Across the top is a 4mm mesh with sides 21cm deep, into which the litter sample was placed. The sample was kept in the centre to retain Haarlov´s passage at the edges. At the sides of the funnels are 4 air vents which at the start of extraction were open. Above each funnel is a fibre-glass hood containing a 60w electric bulb on a dimmer switch. For the first day the hood was suspended approximately 50cm above the funnels with the light at a low level. Subsequently the air vents were closed, the hood was lowered and the light (and therefore heat) increased, these changes took place over a period of several days. For the last 2 days of the 10 day extraction the light was fully on.

These funnels were used to extract animals from the leaf litter layer only. On 5.6.86 four samples of leaf litter were taken randomly from each site. Only the surface litter was taken ie. the previous year´s leaf fall, thus the samples were comparable between the sites. Each sample was 0.25m^{2} in area thus the total area of the sample was 1m^{2}. Due to differential decomposition the samples varied in bulk and weight between the sites. Samples were treated as described above and after 10 days the litter was removed, weighed, volume was recorded and a subjective estimate made of the nature of the leaf litter.

5.2b iii. <u>High gradient extractor at York University</u>.

The apparatus available at York is designed for the extraction of micro-arthropods from small cores of soil.

A soil corer was designed and made which enables cores 64mm in diameter to be divided into sections 30mm deep. The core sub-samples were precisely the size required to slot into the apparatus at York. On 5.6.86 four cores were taken from each site. The cores went down to the top of the mineral soil and were subdivided into sections 30mm deep, as many as necessary, according to the depth of the layers. In this manner the differing depths of leaf litter and humus layers at each site were taken into account. The cores were stored in a cold room over night and then transported to York. The core sub-samples were placed in the extractor in a random order to avoid any errors a clumped distribution might give.

A full description of the extractor is given in Usher & Booth (1984). It is an aluminium box, divided horizontally by an insulated layer with holes punched in it for the small funnels to sit in. The lower layer, under the funnels, is cooled using cold air whilst the upper layer is lit by two fluorescent strip lamps and also heated by a fan heater. The fan circulates the air continuously and the heater is controlled by a thermostat, so that the temperature can be controlled accurately. A temperature gradient is developed and the animals induced to move down the soil cores into collecting vessels under the funnels. Usher & Booth (1984) considered the extractor to be fairly efficient for samples from the Antarctic; there is no reason to suppose it will prove otherwise for similar sized arthropods from British soils.

The temperature of the heated upper section was increased slowly as shown in Table 5.9 and the extraction took a total of 8 days. At the termination of the extraction the glass vials were removed and the cores were weighed.

5.2c Results and discussion.

5.2c i. Manchester Funnels.

The large Manchester funnels, whilst being used to sample only the upper litter layer, do give an impression of the relative abundances of micro-arthropods active on the surface layers of each site. Table 5.10 shows the number of mites and collembola, and the total of both, from each site. These are shown in terms of area ($1m^2$), volume of litter (litre), and dry weight of litter (gram). The mites are further subdivided into Cryptostigmata, Mesostigmata and one species is listed separately, *Damaeus onustus*, a Cryptostigmatid mite which is large, easy to identify and present in fair numbers at all sites except Wetmoor. Figure 5.2 also illustrates this graphically. Although other soil inhabiting animal groups were recognised, the data are

Table 5.9

TEMPERATURE REGIME USED FOR THE YORK EXTRACTOR

Set up mid-morning, 9 June 1987 at room temperature 15°C.

Day	1	2	3	4	5	6	7	8
Temperature of upper section	15	15	20	20	25	30	35	samples removed

Temperature at lower section approximately 5°C.

Table 5.10

RESULTS FROM MANCHESTER TÜLLGRENS (Mites & Collembola)

GROUP		SITES H.W.	P.W.	T.P.	K.G.	K.W.	W.M.	X^2	P
Damaeus onustus	m^2	284	291	190	328	252	0	316.8	<0.001
	p^{-1}	24	32	24	36	15	0	38.3	<0.001
	g^{-1}	0.83	1.115	0.44	0.886	0.653	0	1.17	>0.05 ns
Cryptostigmata	m^2	1416	495	4101	3202	1889	1563	4071	<0.001
	p^{-1}	118	55	513	356	111	156	723	<0.001
	g^{-1}	4.14	1.896	9.493	8.564	4.894	8.098	7.2	>0.05 ns
Mesostigmata	m^2	1811	532	2431	3665	1951	2032	2474	<0.001
	p^{-1}	151	59	304	407	115	203	401.6	<0.001
	g^{-1}	5.295	2.038	5.627	9.905	5.054	10.528	8.11	>0.05 ns
TOTAL Mites	m^2	3227	1027	6532	6867	3840	3595	5754	<0.001
	p^{-1}	269	114	816	763	226	359	1018	<0.001
	g^{-1}	9.436	3.935	15.12	18.559	9.948	18.627	13.5	<0.05
Collembola	m^2	1752	569	869	284	529	916	1604	<0.001
	p^{-1}	146	63	109	31	31	92	132.4	<0.001
	g^{-1}	5.123	2.18	2.012	0.078	1.37	4.746	6.038	>0.05 ns
TOTAL Mites & Collembola	m^2	4979	1596	7401	7151	4369	4511	4522	<0.001
	p^{-1}	415	177	925	795	257	451	875	<0.001
	g^{-1}	14.558	6.115	17.132	19.327	11.319	23.373	12.1	>0.05 ns

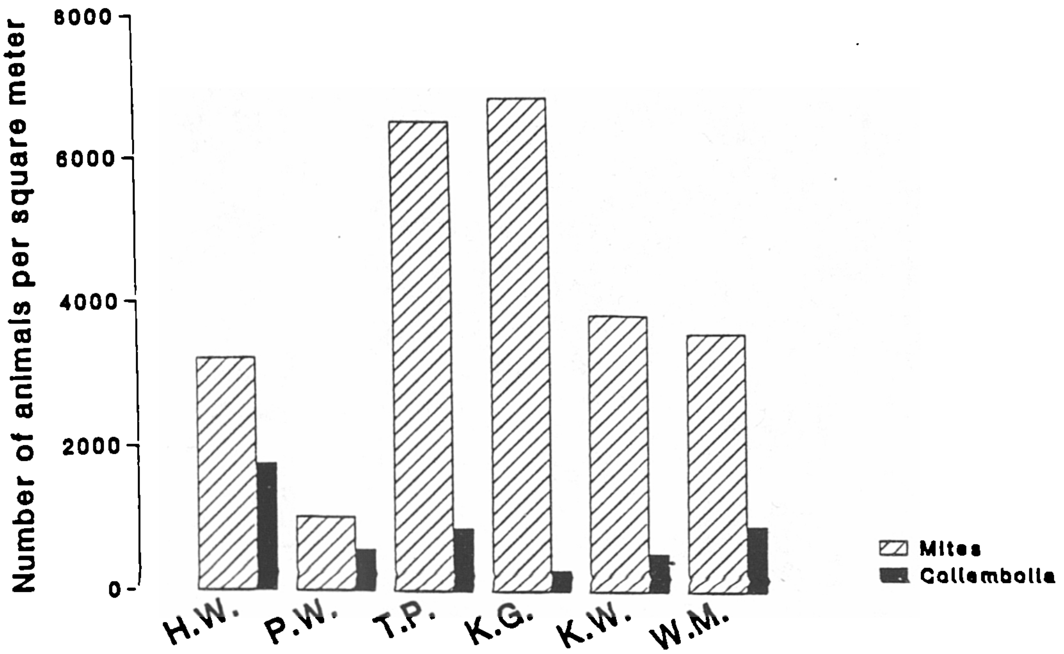
Table 5.10 contd

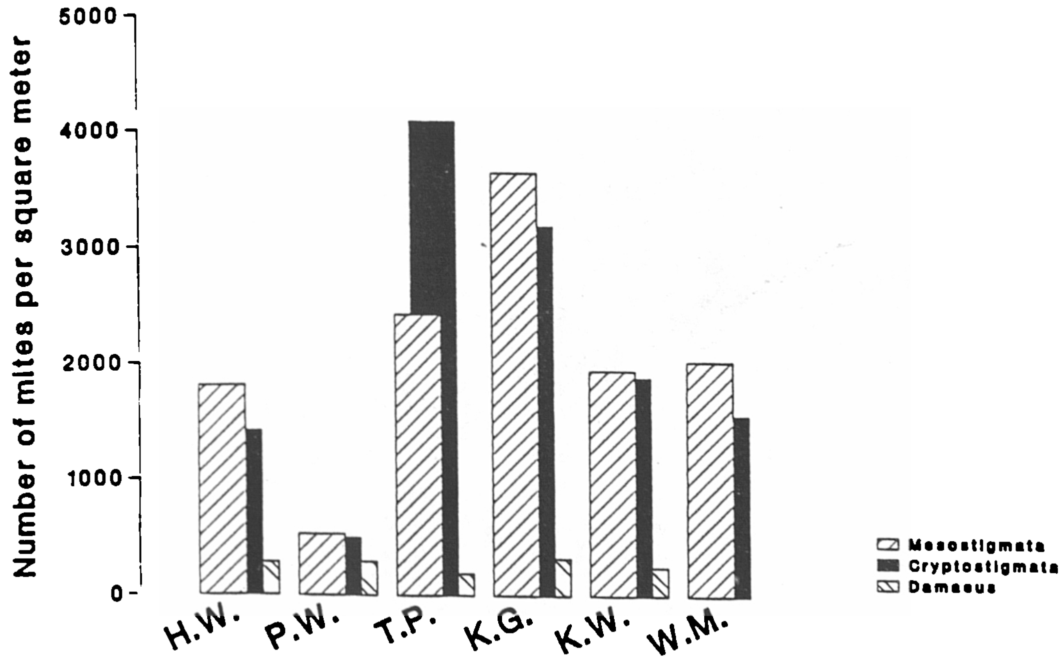
GROUP	SITES						X^2	P
	H.W.	P.W.	T.P.	K.G.	K.W.	W.M.		
CONTENTS								
% WHOLE LEAVES	60	48	20	80	73	94		
% BROKEN LEAVES	50	35	50	15	20	2		
% TWIGS	30	7	30	5	7	4		
% GRASS		10						

Figure 5.2 Bar charts to illustrate the number of microarthropods extracted using the Manchester funnels.

a. Illustrates the number of mites and collembola.

b. Illustrates numbers in broad groupings of mites.


Number of animals per square meter
8000
6000
4000
2000
0
H.W.
P.W.
T.P.
K.G.
K.W.
W.M.
Mites
Collembolla


Number of mites per square meter
5000
4000
3000
2000
1000
0
H.W.
P.W.
T.P.
K.G.
K.W.
W.M.
Mesostigmata
Cryptostigmata
Damaeus

rather sparse, without full lists to species level it was not possible to use these data fully. For each site there was a single sample so a chi squared analysis was used, assuming the expected values to be the same at each site. The results of these can be seen in Table 5.10. In every instance, the number of animals per m^2 and per litre is significantly different. Unfortunately there does not appear to be any obvious trends in the data. The polluted Haw wood commonly falls in the centre, it does not have the fewest micro-arthropods nor the most. It does not seem possible to relate the results to pollution nor to litter depth. Probably more samples from other woods in the area would be beneficial. The main conclusions are simply that the polluted site does not show a large decline in micro-arthropods as reported by Strojan (1978b) and Bengtsson et al. (1983b) and seen in the litter bags of Killham & Wainwright (1981). However all these sites were closer to the sources of pollution than Haw and therefore are likely to suffer higher levels of metals. Also, because the samples taken in this study were just surface leaf litter, they therefore contained lower concentrations than the fermentation and humus layers (see section 4.1). The mites and collembola inhabiting these areas are subject to less severe pollution than those found deeper down. The level of metal concentrations at Haw is however much higher than at any of the other sites.

5.3c ii. York Extractors.

The smaller diameter cores used in the York extractor were necessarily of different depths according to the site sampled. In every instance, samples were deep enough to obtain the entire population living within the surface area of the core. Figures 5.3,a-d illustrate the sharp drop in numbers of animals with depth in the soil and the small number of animals present in the deeper cores. This is consistent with the findings of Macfadyen (1952) who found that not more than 33% of the population occurred below 5cm and Usher (1970) who, when studying the vertical movement of collembola in the soil only took samples to 3cm depth. If the cores taken in this study have yielded every animal within the area of the core, the samples at each site are comparable.

To express the data in a more conventional nature, the figures have been converted to numbers per m^2 of soil surface and per m^3 litter volume. These are shown in Tables 5.11, 5.12 as the mean of the four samples taken at each site (more detailed results are given in appendix 6). Macfadyen (1952) reports,

"One subject on which opinion seems to be divided is whether population figures should be expressed in numbers per unit area or per unit volume of the medium in which they live."

Figure 5.3 Plots to illustrate the number of microarthropods at each depth when sampled using the York extractor.

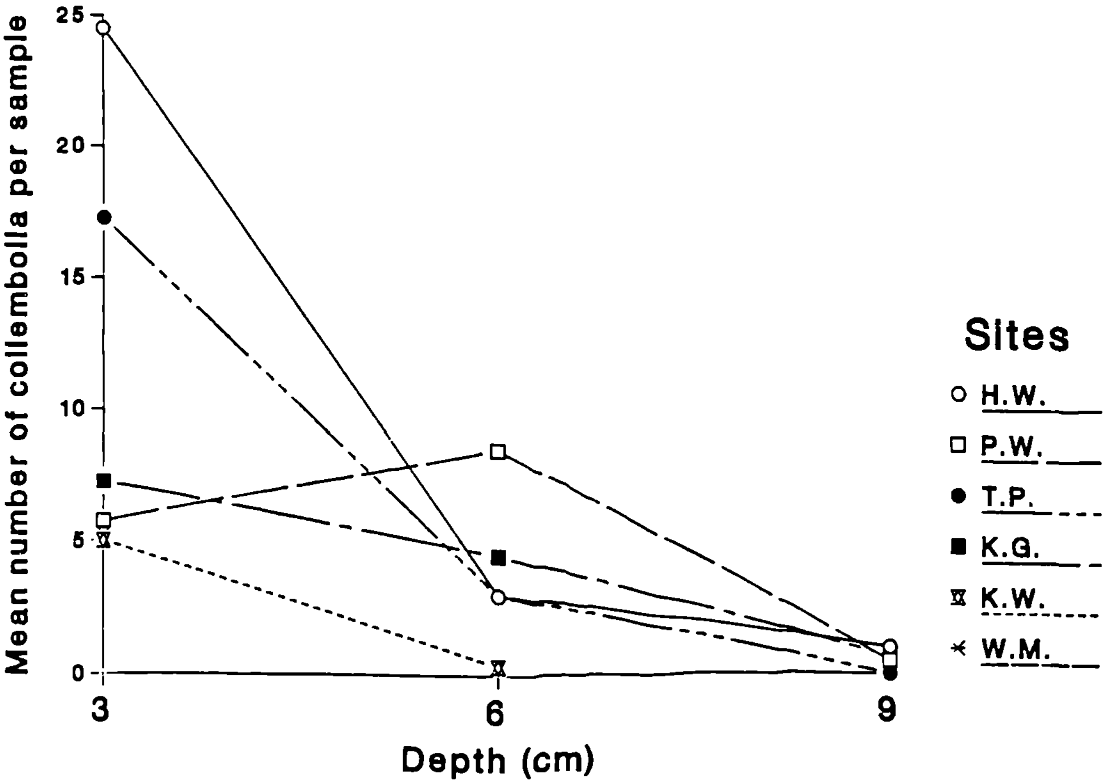
a. Collembola

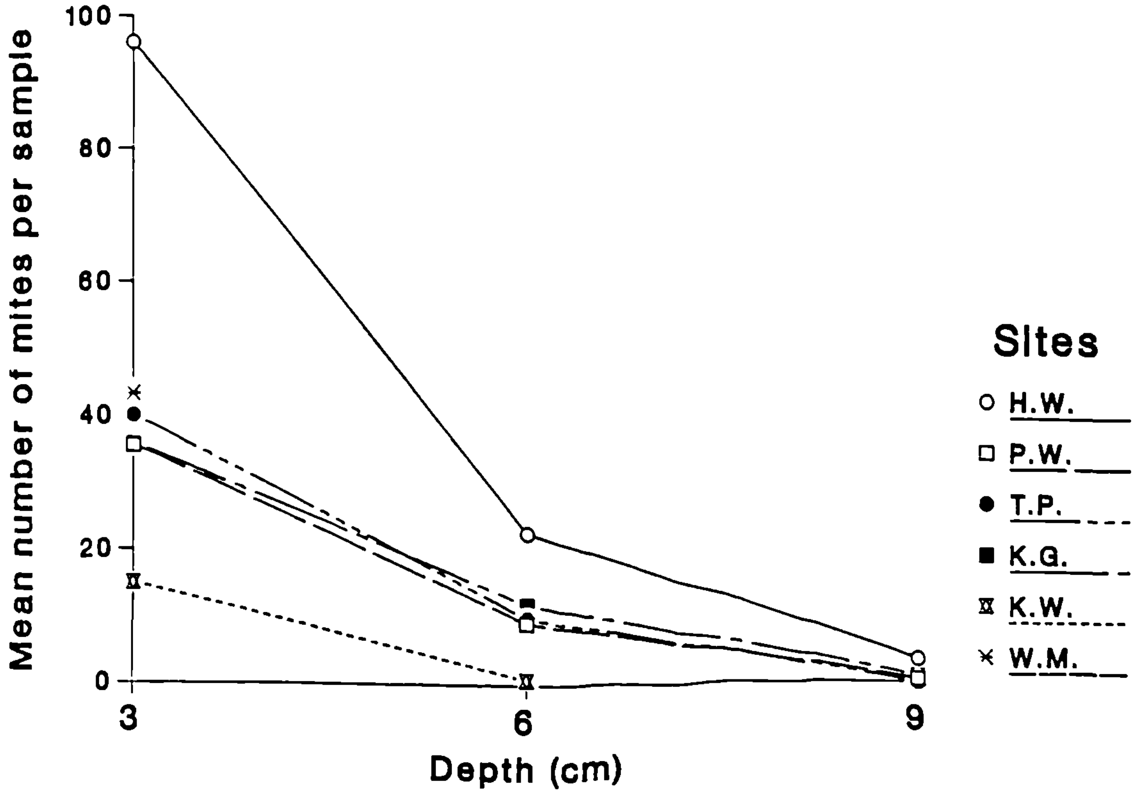
b. Mites

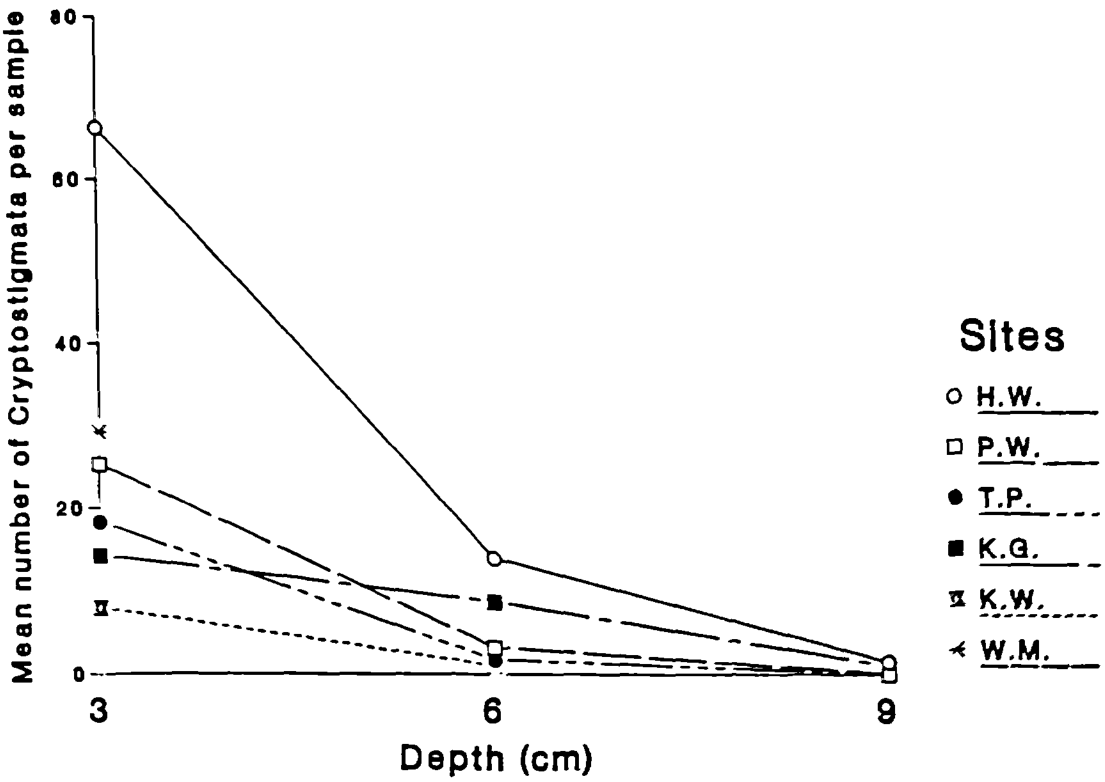
On following page:

c. Cryptostigmatid mites

d. Mesostigmatid mites


Mean number of collembola per sample
25
20
15
10
5
0
3
6
9
Depth (cm)
Sites
H.W.
P.W.
T.P.
K.G.
K.W.
W.M.


Mean number of mites per sample
100
80
60
40
20
0
3
6
9
Depth (cm)
Sites
H.W.
P.W.
T.P.
K.G.
K.W.
W.M.


Mean number of Cryptostigmata per sample
80
60
40
20
0
3
6
9
Depth (cm)
Sites
H.W.
P.W.
T.P.
K.G.
K.W.
W.M.

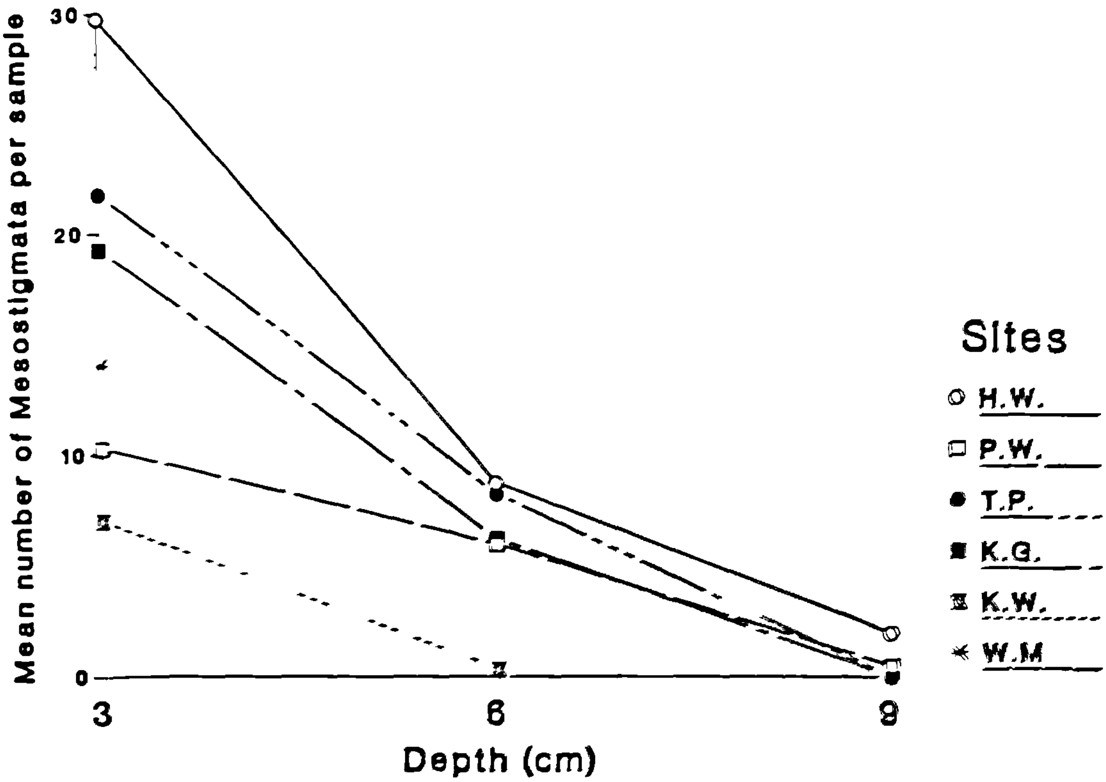

Mean number of Mesostigmata per sample
30
20
10
0
3
6
9
Depth (cm)
Sites
H.W.
P.W.
T.P.
K.G.
K.W.
W.M

Table 5.11

NUMBERS AND MEANS OF MITES PER CORE, AS EXTRACTED USING THE EXTRACTOR AT YORK

SITE	CORE	ABSOLUTE NUMBERS	NUMBERS PER m^2	MEAN PER m^2	NUMBERS PER m^3	MEAN PER m^3
HAW WOOD	A	108	28106	31762 $\pm$ 3457	214206	242456 $\pm$ 26388
	B	154	40010		305420	
	C	94	24422		186427	
	D	133	34554		263771	
PEGWELL WOOD	A	114	29618	11692 $\pm$ 5978	346001	136582 $\pm$ 69839
	B	20	5196		60701	
	C	23	5976		69813	
	D	23	5976		69813	
TOCKINGTON PARK	A	19	4936	12990 $\pm$ 2897	112182	295239 $\pm$ 65843
	B	69	17927		407432	
	C	49	12731		289341	
	D	63	16368		372000	
KINGTON GROVE	A	10	2598	12600 $\pm$ 4307	30209	146517 $\pm$500076
	B	85	22084		256791	
	C	34	8833		102709	
	D	65	16887		196360	
KNAPP WOOD	A	6	1559	4157 $\pm$ 1017	25557	129224 $\pm$ 68576
	B	14	3637		59623	
	C	25	6235		102213	
	D	20	5196		329501	

Table 5.11 contd

SITE	CORE	ABSOLUTE NUMBERS	NUMBERS PER m^2	MEAN PER m^2	NUMBERS PER m^3	MEAN PER m^3
WETMOOR WOOD	A	108	28060	11237 ± 5624	876844	351141 ± 175745
	B	24	6235		194844	
	C	24	6235		194844	
	D	17	4417		138031	
ANALYSIS OF VARIANCE			$F = 4.79$ DF 5/18 $p<0.01$		$F = 1.1$ DF 5/18 $p>0.01$	

Cores A, B, C and D are replicates at each site.

Table 5.12

NUMBERS AND MEANS OF COLLEMBOLA PER CORE, AS EXTRACTED USING THE EXTRACTOR AT YORK

SITE	CORE	ABSOLUTE NUMBERS	NUMBERS PER m^2	MEAN PER m^2	NUMBERS PER m^3	MEAN PER m^3
HAW WOOD	A	61	15848	7404 ± 3241	120977	56523 ± 24737
	B	35	9093		69412	
	C	11	2858		21817	
	D	7	1819		13885	
PEGWELL WOOD	A	6	1559	3767 ± 1728	18213	44010 ± 20188
	B	6	1559		18213	
	C	12	3118		36425	
	D	34	8833		103189	
TOCKINGTON PARK	A	12	3118	5326 ± 1685	70864	121046 ± 38301
	B	20	5196		118091	
	C	11	2858		64954	
	D	39	10132		230273	
KINGTON GROVE	A	17	4417	3182 ± 945	51360	370057 ± 10989
	B	3	779		9058	
	C	10	2598		30209	
	D	19	4936		57395	
KNAPP WOOD	A	0	0	1364 ± 831	0	22360 ± 13621
	B	6	1559		25557	
	C	14	3637		59623	
	D	1	260		4262	

Table 5.12 contd

SITE	CORE	ABSOLUTE NUMBERS	NUMBERS PER m^2	MEAN PER m^2	NUMBERS PER m^3	MEAN PER m^3
WETMOOR WOOD	A	12	3118	)	97437	)
	B	4	1039	) 1364 ± 594	32469	) 42625 ± 18568
	C	2	520	)	16250	)
	D	3	779	)	24344	)
ANALYSIS OF VARIANCE			$F = 1.81$ DF 5/18 $p>0.05$		$F = 2.30$ DF 5/18 $p>0.05$	

Cores A, B, C and D are replicates at each site.

Whilst he himself settled finally for area, other workers have used volume for example Van Der Drift (1951). Haarlov (1960) attempted to measure the interstitial spaces in the soil and express numbers of animals per unit of space. This latter method, although potentially the ideal way of expressing numbers (Macfadyen 1952) is inevitably either very subjective or very time consuming and probably also inaccurate. Macfadyen (1952) is critical of numbers expressed as volume, because the density of animals is greatest at the interface between soil and litter and declined in either direction, hence the interface area will be the same from one site to the next but volume of litter will not. However, the numbers of animals do vary differently with depth at different sites as Figures 5.3 show. Ideally a knowledge of how the numbers of animals vary at each depth, on a smaller scale than 3cm would be better.

The numbers of mites and collembola per m^2 shown in Tables 5.11 and 5.12 can be compared with a table in Macfadyen (1952) where values from a variety of habitats and other workers are presented. The only woodland shown is Van Der Drift's (1951) beech wood (here converted to numbers per m^2) which yielded 3,300 mites and 700 collembola, considerably lower than any of the woodlands in the present study. Macfadyen's Molinia fen (1952) is much richer. The closest comparison number-wise is a grass plain studied by Weis

Fogh, which yielded 18,500 mites and 8,500 collembola m^{-2}. It is unfortunate that no equivalent numbers are available for oak woodland. The variation recorded by Macfadyen (1952) may be as much due to sampling and extraction variation as true differences between the habitat types.

In the six sites studied the numbers of mites exceed the numbers of collembola. In most samples, the number of Cryptostigmatid mites is greater than the number of Mesostigmata. This is to be expected and indicates that detritus and fungal feeding micro-arthropods exceeds the number of predatory animals. Haarlov (1960) has pointed out that although ratios of Oribatid mites and collembola have been discussed many times, the results are contradictory depending upon which species are classed in or out of the groups.

Results of chi squared tests for mites, collembola and both combined are highly significantly different between sites ($p<0.001$). An analysis of variance for these groups for both number per m^2 and per m^3 are shown in Tables 5.11 and 5.12.

One problem with sampling micro-arthropods using small cores is the factor of aggregation. Usher (1969) lists three possible distribution types for collembola in the soil. Of the three, the aggregated type was found in 71.7% of his samples. This aggregation is thought to help in mating in collembola and also be caused by animals seeking pockets of high humidity in both collembola and Cryptostigmatid mites (Verhoef & Nagelkerke 1977, Berthet 1964). With the small number of replicates taken (4), it is quite possible that the presence or absence of aggregations may cause discrepancies in the data. The York data however support that from the Manchester funnels, in that there appears to be differences between the sites, but these are apparently unrelated to the environmental variables studied here.

5.2d General discussion and conclusions.

Metals have been shown to have an effect on collembola. Whilst Joosse & Buker (1979) demonstrated that the majority of lead taken in with the food is either passed out in the faeces or is stored in the intestinal epithelial cells and is lost at ecdysis, effects on life history parameters have been shown. Bengtsson et al. (1983b) fed collembola on fungi with added copper and lead and observed a reduced growth rate and a reduced average maximum size at maturity. Joosse & Verhoef (1987) recorded slower growth rates in animals from lead contaminated soils too. The growth of a population of collembola was monitored by Bengtsson et al. (1985b) over 100 weeks and two generations, a model then extended this for a further period using values obtained for the animals in culture. Extinction was predicted for a population subject to high concentrations of lead, because of the low reproductive rate and the smaller size at maturity which produces lower fecundity. This is perhaps an explanation for the reduced numbers of animals reported by Strojan (1978b). However, oribatid mites and collembola are recorded as eating a wide range of food, such as fungi (Mitchell & Parkinson 1976), algae (Joosse & Buker 1979), general plant material (Anderson 1975) and detritus (Bengtsson et al. 1983b). At Haw there is no apparent reduction in the micro-organisms (Martin et al. 1980) so there is no lack of food for the animals, particularly if, as Bengtsson et al. (1985a) suggest, some species of collembola can change food sources. This is in contrast to the situation in Sweden, where Bengtsson & Rundgren (1982) report reduced fungal mycelial lengths of micro-fungi close to the brass mill. It is possible that fungi at Haw do not accumulate metals, but this is unlikely judging from Bengtsson et al. (1985b). It is also possible that the animals, or at least some of them have become tolerant to heavy metals. It should be noted that all the experimental studies have been based on only a handful of species and are predominantly concerned with lead. Van Straalen & Van Wensem (1986) when looking at a range of invertebrates did find however, that the lowest concentrations of zinc and cadmium were in three species of collembola.

The distribution of mites and collembola at Haw shows a significant predominance in the top 3cm (oneway analysis of variance $F=12.63$ $p<0.01$). It is thus possible that freshly fallen litter is decomposed with the help of mites and collembola, but as it becomes lower in the profile, it is too contaminated (see Figure 4.2) and is avoided, hence the lower layers build up in volume.

Unfortunately, the limitations of time and facilities in this study have produced more questions than they have answered. More replicates would have helped to avoid problems of patchy distributions. The species composition is important to establish; have a few species overcome the pollution problem or is it more widespread? The age structure within the species present needs investigation. Is the growth rate slow and is fecundity lower at the polluted sites? Finally an unanswerable question, did the numbers drop at some point in the past and are they now on the increase? If the fungal population is good and the mite and collembola populations are healthy (and the data does not show otherwise) the part played by the micro-arthropods in the polluted site does not seem in jeopardy.

5.2e SUMMARY.

1. Micro-arthropod populations at each site were sampled by two sets of Tullgren type apparatus. One used only the fresh litter layers, the other measured animals in a core which included all the litter layers above the mineral soil.

2. When sampling the litter layer only, differences in numbers of mites and collembola were found between the sites. This could not be related to degree of pollution.

3. When sampling down to the mineral soil, similar results were obtained. Differences in numbers of mites and collembola (both in numbers per unit area and per unit volume) occurred between the sites, but Haw did not show reduced numbers.

4. There is no evidence for any relationship between numbers of microarthropods and metal pollution. Not absolute metal concentrations, distance from smelter nor litter depth produced a response in numbers of animals.

5. Other workers have shown that collembola are susceptible to lead pollution, it is possible that Haw is not contaminated enough to show these effects.

6. Topics for future interest are presented.

5.3 Laboratory experiments using millipedes.

5.3a Introduction.

Millipedes are important decomposers found in many habitats including woodlands. Studies of them as adults and as juveniles are important and informative. Pitfall trapping data from the most polluted site revealed that whilst two fairly common species were present, *G. marginata* and *P. angustus*, another ubiquitous species, *T. niger*, was absent. In order to help to understand this occurrence, laboratory feeding trials have been undertaken.

5.3b *Tachypodoiulus niger* adults.

5.3b i. Methods.

Adult animals were collected from Knapp wood, a clean site supporting large numbers of this species, on 27.6.86. On 30.6.86 individuals were separated from their own leaf litter and placed in petri dishes, one per dish. In each petri dish was placed a disk of filter paper moistened with deionised water and three pieces of field maple (*Acer campestre*) leaf. Half the dishes contained leaves from Wetmoor (clean) and half from Haw (contaminated). The leaves were analysed for metal concentrations as described in section 3.1d. All the dishes were then placed in a covered plastic tank with damp absorbent paper on the base and kept at room temperature. Periodically dishes were checked, extra food added if necessary and dead animals and faeces removed. After 10 weeks the experiment was terminated. All animals were sexed and the stadial age determined using the ocular field method (Vachon 1947 and Saudray 1953). Both faeces and animals were dried, weighed and analysed for metal levels.

5.3b ii. Results: metal concentrations in the leaves.

The metal concentrations in the field maple leaves used are shown in Table 5.13. Twosample t-tests were calculated and for cadmium, lead and zinc there were significant differences between the concentrations of metals in leaves from Haw and from Wetmoor. Although the values for copper were higher for Haw this difference was not significant. However, it is probable that copper is the least important metal of the four in causing physiological problems in animals, in the concentrations in which it is found at the sites. Unfortunately the leaves were analysed well after the termination of all the relevant experiments. Despite being kept in a cold store the leaves from Wetmoor had decomposed considerably whereas those from Haw appeared 'untouched'. It is possible that the increased decomposer activity in the Wetmoor leaves has had the effect of concentrating the metals present. This effect has been shown by Nilsson (1972) in spruce litter. However, by comparing the values with those found in leaves used by Hopkin & Martin (1984a) Table 5.14 it can be seen that

Table 5.13

CONCENTRATIONS OF METALS IN THE ACER CAMPESTRE LEAVES USED IN THE FEEDING EXPERIMENTS (Mean ± S.E)

	HAW LEAVES	WETMOOR LEAVES	t	df	p
Cd	3.47 ± 0.64	0.99 ± 0.11	3.83	4.2	0.019*
Cu	32.9 ± 8.8	11.97 ± 0.96	2.37	4.1	0.077ns
Pb	122.5 ± 10	24.4 ± 2.6	9.17	3.4	0.0027 **
Zn	232.5 ± 43	89.3 ± 8.3	3.24	4.3	0.032*

Table 5.14

COMPARISON OF METAL CONCENTRATIONS IN LEAVES USED IN THIS STUDY AND THOSE USED BY *HOPKIN AND MARTIN* (1984)

	METALS (Means only)			
SITE/RECORDER	CADMIUM	COPPER	LEAD	ZINC
HAW				
HOPKIN & MARTIN	5.7	42.0	502	807
READ	3.5	32.8	122.5	232.5
WETMOOR				
HOPKIN & MARTIN	0.6	12.8	37.1	72.1
READ	0.99	11.9	24.4	89.3
RATIO WETMOOR:HAW				
HOPKIN & MARTIN	1:9.5	1:3.3	1:13.5	1:11.2
READ	1:3.5	1:2.76	1:5.2	1:2.6

levels of all four metals are lower than those in 1984. This may be due to a difference in the time of year of collection, or a genuine reduction in deposited metals at the polluted site.

5.3b iii. Results and discussion of experiment.

After 10 weeks, 6 of the 7 animals fed on Wetmoor leaves were alive and 5 of the 8 fed on Haw leaves. The eighth Haw fed millipede was the only male represented and so is ignored in the following analysis in case of differences between the sexes. The stadial distribution was very similar in both groups, ranging from VIII to XI. Table 5.15 indicates the mean metal concentrations for each group of animals. Also presented is the mean dry weight of faeces produced (giving an indication of the quantity eaten) and faeces per day which corrects for the different experimental life span of some of the animals. The t values are for t-tests between the two groups and it can be seen that none of them are significant at the 5% level. Only lead is close to significance at 10%.

Metal concentrations in the faeces are shown in Table 5.16 together with the relevant t-tests. In contrast to the animals themselves there are significant differences between the groups for all four metals. These results suggest that the adult animals are not particularly affected by the polluted litter. They eat a similar quantity to those fed

Table 5.15

METAL CONCENTRATIONS (μg/g) AND FAECES DRY WEIGHTS IN ADULT T. NIGER FED ON DIFFERENT LITTER TYPES (mean ± S.E.)

	WETMOOR FED	HAW FED	t	df	p
n	6	7			
Cadmium	1.06 ± 0.36	3.09 ± 1.36	-1.34	11	0.21 ns
Copper	179.7 ± 12.4	217.2 ± 26.2	-1.22	11	0.25 ns
Lead	0.51 ± 0.17	3.28 ± 1.19	-2.31	11	0.06 ns
Zinc	336.6 ± 96.8	458.0 ± 74.4	-1.01	11	0.33 ns
Mean Faeces Weight (g)	40.60 ± 14.1	10.28 ± 3.3	2.09	5.5	0.091 ns
Mean Faeces day (g)	0.83 ± 0.22	0.28 ± 0.14	2.07	8.9	0.072 ns

Table 5.16

METAL CONCENTRATIONS (μg/g) IN FAECES FROM ADULT T. NIGER FED ON DIFFERENT LITTER TYPES (mean ± S.E.)

	WETMOOR FED	HAW FED	t	df	p
n	27	28			
Cadmium	1.57 ± 0.2	94.0 ± 2.41	-3.24	27.4	0.0032 **
Copper	70.0 ± 11.2	121.3 ± 21.1	-2.15	41.1	0.038 *
Lead	23.47 ± 3.84	388.0 ± 107	-3.39	27.1	0.0021 **
Zinc	226.4 ± 30.7	721.0 ± 135	-3.59	29.8	0.0012 *

on clean litter and do not accumulate the metals which pass out in the faeces. This is in contrast to the results shown by Hopkin _et al_. (1985) using adult _Glomeris marginata_ and demonstrates the variability between different species. However it is possible that the low number of replicates may have contributed to the differences between metal levels in the animals themselves. It does not help to explain why _T. niger_ is absent from polluted sites.

A similar experiment was carried out using _Ophyiulus pilosus_, due to the small number of animals involved, the results are shown in appendix 6.

5.3c _Tachypodoiulus niger juveniles_.

5.3c i. _Methods_.

Adult _T. niger_ were collected from Knapp wood early in 1986 and were kept as pairs in plastic sandwich boxes containing their own litter. At the end of May, two females made nests and laid eggs which later hatched. When the young reached stadium II they were separated and placed in petri dishes as in experiment 1. Dishes again contained damp filter paper and _Acer campestre_ leaves from either Wetmoor or Haw. Six individuals were placed in each dish. Each family had young placed on both litter types. A small number of animals were dried for analysis at this stage. This experiment ran for 14 weeks. Any animals which died were removed, aged and dried for analysis. At the end any animals still alive were treated likewise. Early attempts to remove faeces were abandoned due to their small size.

5.3c ii. Results and discussion.

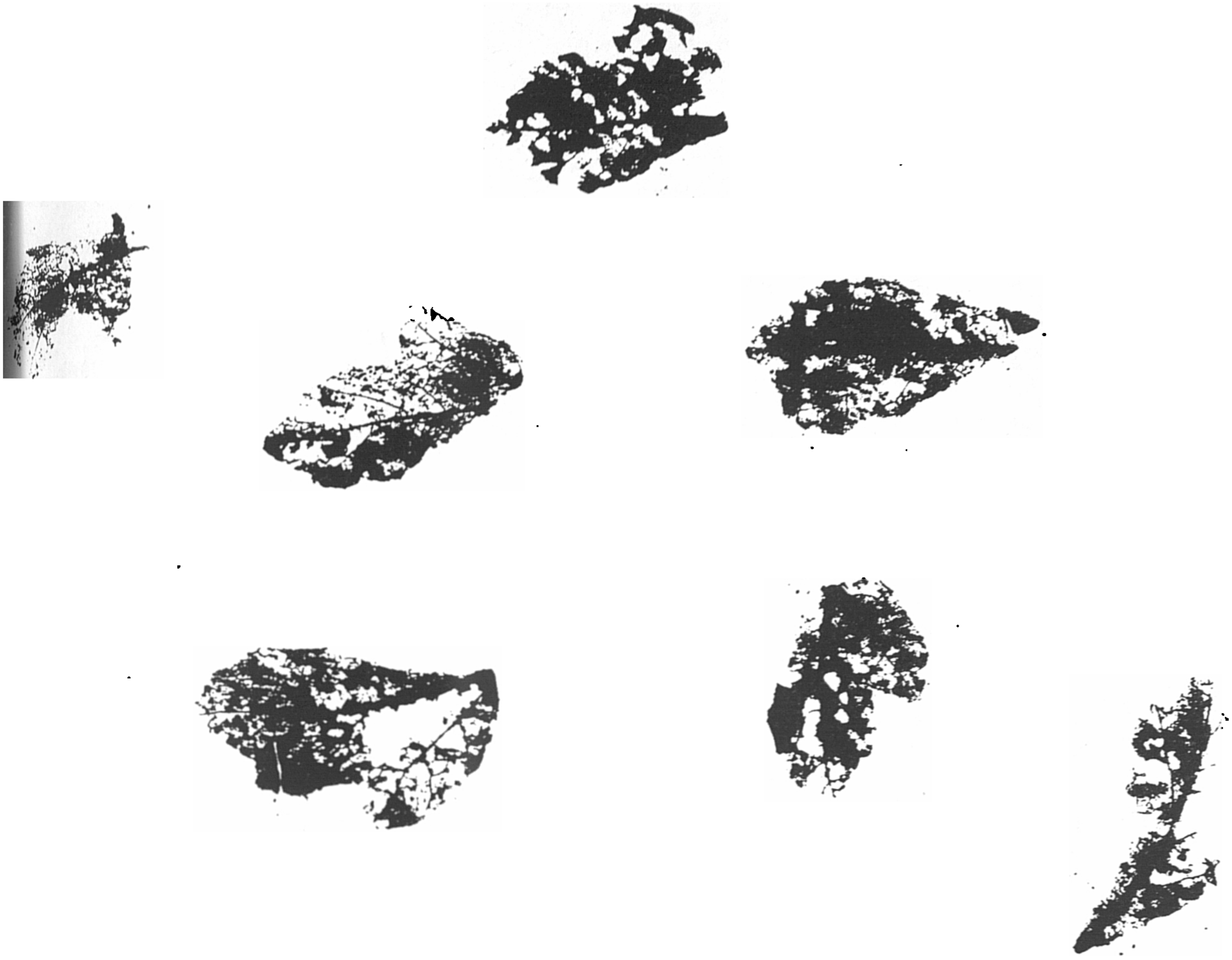
It was immediately obvious that animals fed on leaves from Wetmoor ate more than animals from Haw, Figure 5.4 illustrates this feature. Individuals on clean litter also had a higher survival rate. Of the initial 42 animals fed on clean litter 17 were alive at the end of the experiment in comparison to 1 out of 42 fed on contaminated litter. Figure 5.5 shows the survival rates graphically. A Mann-Whitney U test was calculated to compare the median date of death for each group and this was significant ($p<0.001$). Of the animals alive and growing, the growth rate was quicker in those animals feeding on clean litter. 10 individuals reached stadium VI on Wetmoor litter whereas the oldest stadium attained in the Haw fed animals was IV. Figure 5.6 shows the stadial age at death (or termination of the experiment) to be significantly different between the sites ($p<0.001$). This is also illustrated by dry weight shown in Table 5.17 which shows statistically significantly differences for the two groups.

When treating the Haw fed and Wetmoor fed animals separately, no significant differences were found between the two families in weight, or any of the metal

Figure 5.4 A photocopy of *Acer campestre* leaves after having been fed to juvenile *Tachypodoiuls niger*.

Top leaves were from Wetmoor (clean).

Bottom leaves were from Haw wood (contaminated).



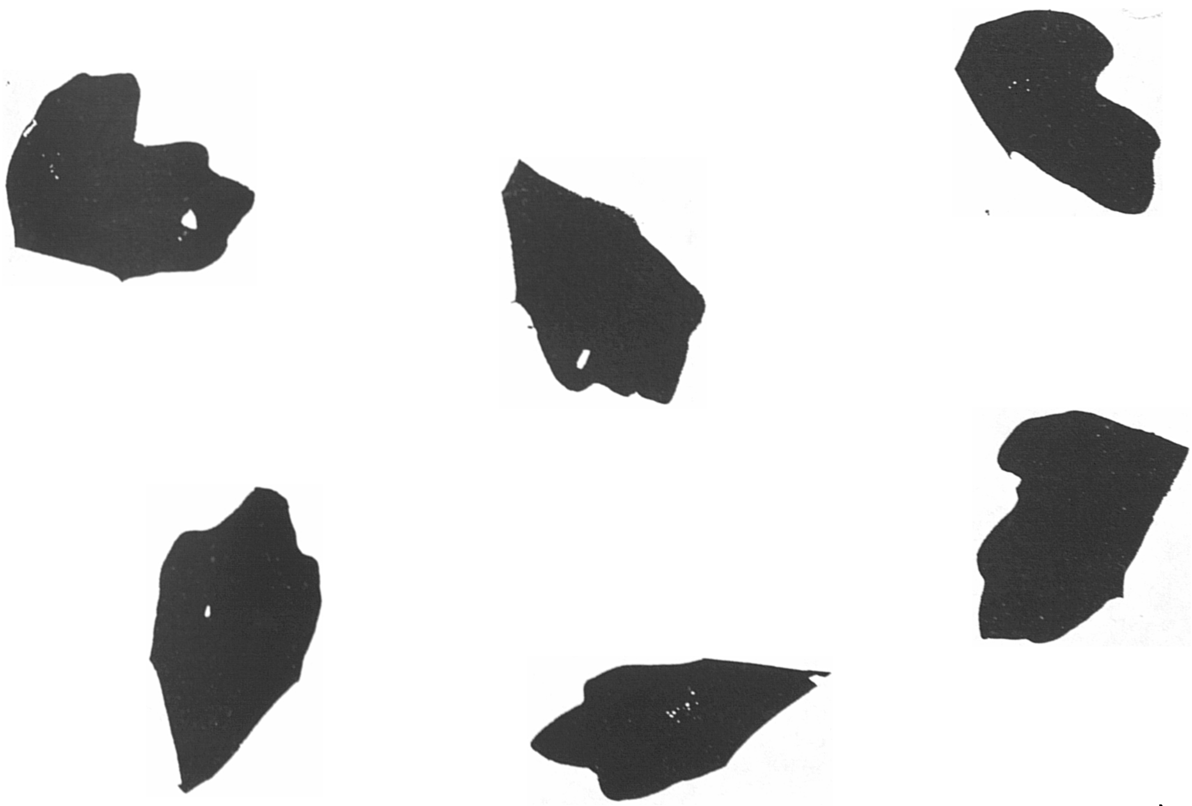
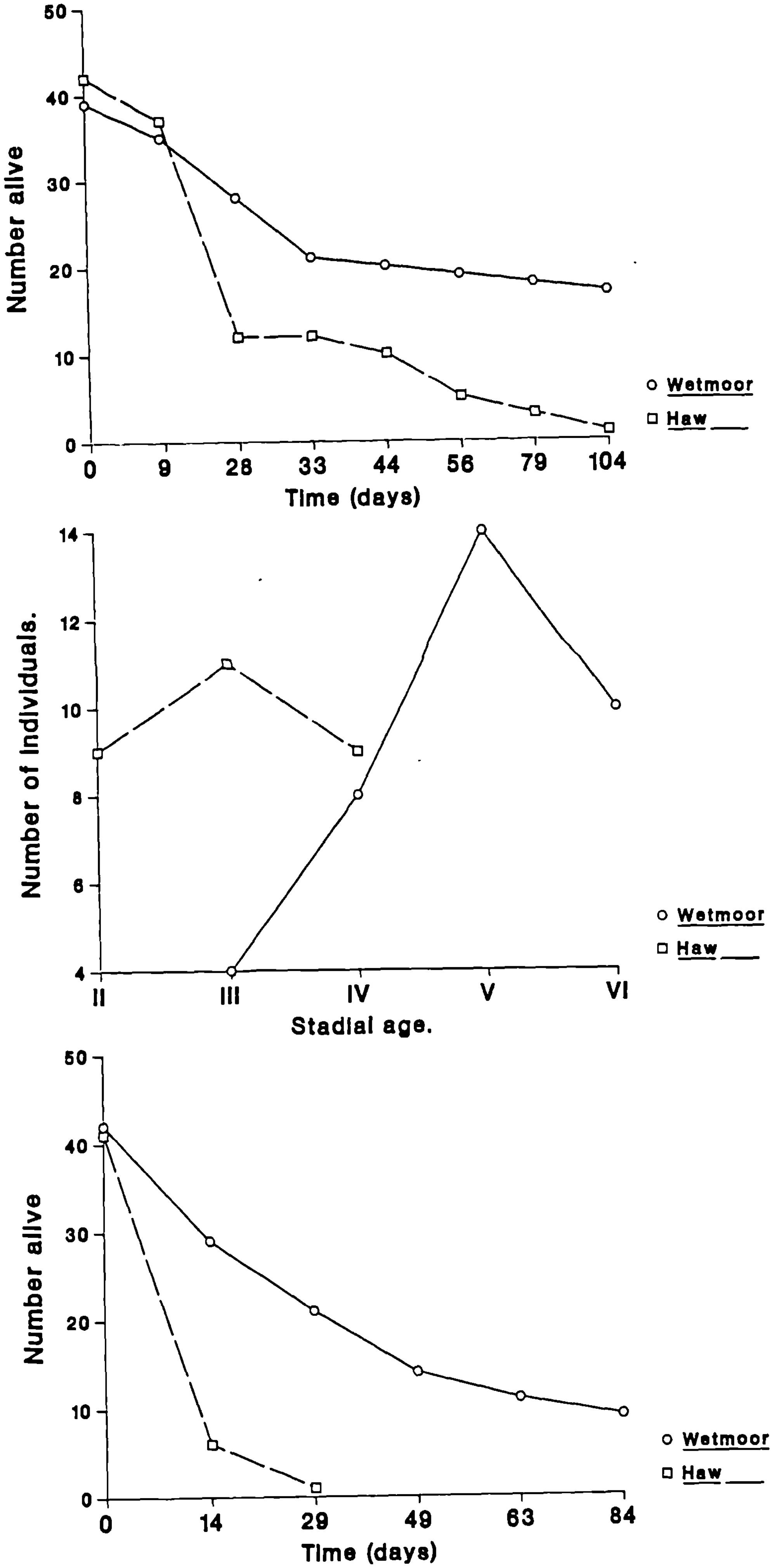


Figure 5.5 Survivorship curves for juvenile *Tachypodoiulus niger* fed on leaves from Wetmoor (clean) and Haw (contaminated).

Figure 5.6 Stadial age at death (or termination) of juvenile *Tachypodoiulus niger* fed on leaves from Wetmoor and Haw wood.

Figure 5.7 Survivorship curves for juvenile *Glomeris marginata* fed on leaves from Wetmoor and Haw wood.



50
40
30
20
10
0
Number alive
0
9
28
33
44
56
79
104
Time (days)
o Wetmoor
□ Haw
14
12
10
8
6
4
Number of Individuals.
II
III
IV
V
VI
Stadial age.
o Wetmoor
□ Haw
50
40
30
20
10
0
Number alive
0
14
29
49
63
84
Time (days)
o Wetmoor
□ Haw

Table 5.17

DRY WEIGHTS (mg) AND METAL CONCENTRATIONS ($\mu g/g$) IN JUVENILE T. NIGER REARED ON DIFFERENT LITTER TYPES. ALSO SHOWN ARE THE RESULTS OF T - TESTS BETWEEN THEM AND THE VALUES FOR 4 ANIMALS AT THE START OF THE EXPERIMENT

	Wetmoor fed	Haw fed	t	df	p	Originals
n	36	34				4
Weight	0.86 $\pm$ 0.11	0.18 $\pm$ 0.022	6.17	39	<0.001	0.109 $\pm$ 0.0219
Cadmium	0.75 $\pm$ 0.11	8.72 $\pm$ 1.45	-5.47	33.4	<0.001	1.83 $\pm$ 0.67
Copper	200 $\pm$ 12.3	569.5 $\pm$ 40.2	-8.77	39.1	<0.001	157.3 $\pm$ 33.6
Lead	22.2 $\pm$ 7.07	228.8 $\pm$ 36.3	-5.59	35.5	<0.001	40.6 $\pm$ 18.3
Zinc	607.6 $\pm$ 38.5	1363.7 $\pm$ 96.2	-7.29	43.4	<0.001	1032.0 $\pm$ 127.0

concentrations. Thus it is justified to lump the two families together. The metal concentrations recorded in the animals are shown in Table 5.17. All of these are significantly higher in the Haw fed animals. Original values of the stadium II juveniles before the treatments are shown in column 7. Although the sample size for this group of animals is very low, the values are mostly in the order of the Wetmoor fed group. The exception, zinc, has a large standard error. Hopkin & Martin (1984a) undertook similar experiments using juvenile *Oniscus asellus* (Isopoda) and obtained similar results. It appears from this experiment that juveniles are far more susceptible to contaminated litter than the adults.

5.3d *Glomeris marginata juveniles*.

Juvenile *G. marginata* bred from a single female originating from Knapp wood were treated in a similar manner to the *T. niger*. The differences between the two experiments were that stadial age, which is determined in this species by counting the number of segments (Heath *et al*. 1974), proved too difficult to ascertain. Also the metal concentrations were not analysed in the animals prior to the experiment.

Of the 42 animals on each food type, 9 were alive at termination on the Wetmoor litter, whilst none survived more than 7 weeks when fed Haw litter. Figure 5.7 shows survival curves for the animals on the different diets. A Mann-

Whitney test again showed a significant difference between the two groups ($p<0.001$). Table 5.18 indicates mean weights and metal concentrations for the two groups. The weights were again significantly different, evidence of the differential growth and survivorship. Cadmium and zinc concentrations were significantly higher in animals fed on polluted litter, however copper and lead were not. The means for lead are considerably different and the values for Haw show a large standard error, indicating that there may be differences between individuals in lead uptake. This, together with the non-significant result for copper indicates that this species may have the potential for survival in polluted environments. Due to the low survival rate it seems surprising that this species is now found in large numbers at Haw. Investigation of individuals from this site may reveal increased tolerance to levels of pollution found at the site.

5.3e *Polydesmus angustus juveniles*.

Two female *P. angustus* from Knapp wood, when kept on their own litter produced young in May 1986. When the juveniles had 9 segments (II stadium, see Blower 1985 for details) they were separated out onto petri dishes as before, this time with 10 per dish and 5 dishes for each treatment. The experiment ran for 15 weeks. The mortality was very high on both litter types, only 5 surviving from the clean litter to reach stadium V (with 17 segments) and one V stadium

Table 5.18

DRY WEIGHTS (mg) AND METAL CONCENTRATIONS (μg/g) OF JUVENILE G. MARGINATA REARED ON DIFFERENT LITTER TYPES

	WETMOOR FED	HAW FED	t	df	p
n	36	39			
Weight	0.36±0.059	0.21±0.013	2.59	38.2	0.014*
Cadmium	8.83±1.06	11.54±0.84	-2.02	67.5	0.048*
Copper	75.9±13.0	97.7±15.9	-1.06	69.9	0.29ns
Lead	62.0±14.6	185.0±73.6	-1.64	39.9	0.11ns
Zinc	827.2±68.9	1052.3±59.4	-2.48	68.5	0.016*

surviving from the polluted litter. Unfortunately due to the tiny size of the animals and their almost transparent colour, a large number of dead animals were never recovered. Table 5.19 gives the results obtained for those individuals available for analysis and for 5 originals kept back at the beginning.

Oneway analysis of variance was calculated, comparing the three groups for each metal concentration. Significant results were obtained for all four metals. Using t-tests to compare between the Wetmoor and Haw fed animals only copper and zinc gave significant values. Zinc was also significant between the two groups of G. marginata which indicates that perhaps, although an essential element, it is causing more problems to the animals than the other metals.

P. angustus was found in high numbers at the most polluted trapping site and further studies of this species in the laboratory may prove interesting. It may be more profitable to start with slightly older animals or alternatively try different conditions for keeping this species.

5.3f General discussion and conclusions.

It appears that adults of T. niger are able to withstand high levels of metal pollution at least for short periods of time. Juveniles of this species and others seem to be very

Table 5.19

DRY WEIGHTS (mg) AND METAL CONCENTRATIONS (μg/g) OF JUVENILE P. ANGUSTUS

REARED ON DIFFERENT LITTER TYPES

	WETMOOR FED	HAW FED	ORIGINALS (Before feeding)	F	p
n	10	7	5		
Weight	0.234 ± 0.056	0.091 ± 0.0405	0.0734 ± 0.0046	3.32	0.05 ns
Cadmium	2.37 ± 0.51^{a}	9.98 ± 3.1^{b}	1.55 ± 0.71^{a}	6.14	0.05 *
Copper	328.6 ± 34.8^{a}	1351 ± 279^{b}	333.1 ± 37.2^{a}	12.93	0.01 **
Lead	53.6 ± 22.9^{a}	621 ± 286^{b}	59.2 ± 25.2^{a}	3.84	0.05 *
Zinc	1497 ± 229^{a}	3780 ± 534^{b}	1098 ± 93.9^{a}	15.99	0.01 **

Superscripts show the results of subsequent t-tests.

Means showing the same letter are not significantly different from each other.

susceptible to contamination. The levels of metals build up in their tissues, which can also be seen as effects on the survival and growth rates. In some animals increased levels of copper and zinc can interfere with ecdysis, increasing the length of time between moults, this leads to a slower growth rate (Sheehan 1984a). The polluted leaves may also be distasteful in some way, reducing the intake of food. The chief problem in contaminated areas is not then, surviving as an adult, but surviving as a juvenile.

It is easy to see why species are absent from areas of high pollution but less easy to discover why others are present. Young *P. angustus* and *G. marginata* suffered high mortality in these experiments. Laboratory conditions do not equate to conditions in the field and certainly with *P. angustus* survival was low in those individuals fed on clean litter. Mortality may not be as high in polluted regions as might be predicted from this experiment where animals can be more selective in what they eat. *G. marginatus* showed an increase in levels of two metals (cadmium and zinc) but not the other two (copper and lead). The potential for survival may be greater because of this than in *T. niger*. Hopkin *et al*. (1985) showed that *G. marginata* did not assimilate lead with which these findings agree. Further studies using juveniles derived from polluted areas are necessary to clarify the picture. Whilst Hopkin & Martin (1984a) found that levels of metals in newly born *Oniscus asellus* from a polluted site were comparable to those in animals originating from clean sites, data on millipedes are obviously required. Until they are obtained, it is not possible to be clear as to whether these species can control the uptake of metals as suggested for the woodlice *Porcellio scaber* (Wieser 1979), or whether animals in polluted sites have evolved to be more tolerant as in *Asellus aquaticus* (Fraser 1980).

An interesting feature of the millipede fauna of Haw wood is the presence of *Chordeuma proximum*. This species appears to have no problem in surviving as juveniles, as young animals were caught during the trapping. Attempts to keep this species in the laboratory even as adults, proved unsuccessful since the level of humidity appears to be critical. Preliminary analysis of 4 individuals from Haw for metal content showed the levels to be comparable to those obtained for *G. marginata* (Table 5.20) with the exception of copper, which was approximately 10 times higher. This species has a greater capacity for the evolution of tolerance than some of the other species as it appears to have a shorter life cycle of only one year (Read in manuscript). *P. angustus* takes two years to reach maturity (Blower 1969) whereas *T. niger* and *G. marginata* take 3 and 3-4 years respectively to reach maturity (Blower & Fairhurst 1968, Heath *et al.* 1974). More information on *C. proximum* may be invaluable particularly in view of its patchy distribution at present (Blower 1985).

Table 5.20

METAL CONCENTRATIONS IN CHORDEUMA PROXIMUM AND GLOMERIS MARGINATA FROM HAW WOOD

	CHOR_DEUMA PROXIMUM	GLOMERIS MARGINATA
n	4	66
DRY WEIGHT	3.172 ± 0.515	17.98 ± 1.9
Cd	31.7 ± 16.8	26.49 ± 0.74
Cu	723 ± 102	71.53 ± 1.1
Pb	47.63 ± 8.26	30.1 ± 2.91
Zn	613 ± 34	714 ± 10.9

5.3g SUMMARY.

1. With the limited data available it appears that adult *T. niger* when fed on polluted leaves do not appear to eat less, nor accumulate more metals than those fed on clean litter. Those fed on contaminated litter did however excrete significantly higher concentrations of metals in their faeces.

2. Juvenile *T. niger* originating from a clean site, when fed on polluted litter show slower growth, reduced survivorship and higher metal concentrations in their tissues than those fed on clean litter.

3. Juvenile *G. marginata* in similar circumstances show reduced survivorship and higher concentrations of cadmium and zinc.

4. Juvenile *P. angustus* are less easily kept in the laboratory. Animals fed on polluted litter had a reduced survival and suffer elevated concentrations copper and zinc.

5. More information is required on juvenile animals originating from contaminated sites and on *C. proximum* adults as well as juveniles.

6. Life histories of some species may predispose them to adaptations to polluted areas.

5.4 Measurement of metal concentrations in two active decomposer species.

5.4a The effect of preservatives on metal concentrations in *Oniscus asellus*.

5.4a i. Introduction.

There are occasions when animals which are to be analysed for metal concentration cannot be dealt with immediately. Methods of storage in the past have included drying or freezing, or use of some chemical preservative. Many methods of catching invertebrates also include preservatives, for example pitfall trapping. In previous studies animals used for analysis have been caught in formalin filled pitfall traps (Roberts *et al*. 1978), caught in formalin and shaken in 70% ethanol (Williamson & Evans 1972) and animals caught in formalin but added to animals caught fresh (Wade et al. 1980). Hunter *et al*. (1987b) caught animals in formalin before analysis and comments that this did not cause leaching of copper or cadmium from the specimens. In the present study, animals were caught in traps containing formalin and were transferred to 70% ethanol for identification before analysis. It is therefore important to establish any effects this treatment might have on the metal concentrations.

5.4a ii. Methods.

Specimens of the woodlouse *Oniscus asellus* were collected by hand from 2 sites within Haw wood in October 1986. One site was close to the pitfall trapping grid, at the end of the wood nearest to the smelter, the other was at the end farthest away from the smelter.

The animals from each site were divided into groups of ten and treated in one of four ways. One group was dried in the collecting tubes, one was frozen and another was placed in 70% ethanol. The final group was put into 4% formalin for two weeks and then transferred to ethanol, in order to simulate the pitfall trapping procedure. Specimens were maintained in these states until March 1987 when they were oven dried at 80^{o}C and digested individually in nitric acid as described in section 3.1d.

5.4a iii. Results and discussion.

The results of the chemical analyses are shown in Table 5.21. Analysis of variance were calculated for each metal and site separately. For site 1, that nearest the smelter, significant results were obtained for the dry weight and lead concentrations. However for site 2, significant results were obtained for all the metals except zinc. Fixed range tests were calculated for situations where significant

Table 5.21

METAL CONCENTRATIONS ($\mu g g^{-1}$) IN ONISCUS ASELLUS FROM TWO SITES WITHIN HAW WOOD, SUBJECTED TO DIFFERENT PRESERVATIVE TREATMENTS

METAL/	SITE	TREATMENTS					
		DRY	FROZEN	ALCOHOL	FORMALIN/ALCOHOL	F	p
Cd	1	185.8 ± 10.6	190.8 ± 12.3	200.7 ± 20.2	220.2 ± 20.1	0.86	0.05ns
	2	234.2 ± 21.9^{b}	167.97 ± 13.9^{a}	194.0 ± 8.19^{ab}	165.2 ± 17.8^{ab}	3.02	0.05 *
Cu	1	802.7 ± 43	763.0 ± 47.2	733.3 ± 67.4	640.5 ± 49.7	1.72	0.05ns
	2	926.4 ± 73.1^{b}	567.1 ± 57.6^{a}	535.8 ± 73.6^{a}	474.8 ± 47.2^{a}	10.36	0.01 **
Pb	1	515.0 ± 51.3^{a}	453.6 ± 39.5^{a}	540.9 ± 48.4^{a}	698.8 ± 69.0^{b}	3.86	0.05 *
	2	744.7 ± 73.3^{b}	532.6 ± 44.9^{a}	571.1 ± 34.2^{a}	528.3 ± 42^{a}	3.91	0.05 *
Zn	1	618.2 ± 56.7	638.8 ± 41.1	643.6 ± 95.1	747.6 ± 146	0.38	0.05ns
	2	908.2 ± 186	683.6 ± 76.4	719.3 ± 40.1	738.0 ± 68.0	0.75	0.05ns

Table 5.21contd

METAL/SITE	TREATMENTS				F	p
	DRY	FROZEN	ALCOHOL	FORMALIN/ALCOHOL		
DRY WEIGHT 1	14.53 ± 1.01^{ab}	17.62 ± 2.52^{b}	13.01 ± 0.79^{ab}	10.54 ± 0.91^{a}	3.96	0.05 *
2	13.14 ± 1.77	17.53 ± 1.87	15.96 ± 1.75	15.45 ± 0.82	1.19	0.05ns

Degrees of Freedom 3/36.

Site 1 is nearer to the smelter than site 2. Superscript letters show the results of a fixed range test on significant analysis of variance. Any two means showing the same letter are not significantly different.

results were obtained. In three instances, the dry treatment gave significantly higher metal concentration than at least one of the other treatments. However the lead value from the formalin and alcohol treatment of site 1 is significantly higher than the other treatments. The differences between the treatment are not consistent, even for one metal, as the results for lead show.

The animals which were dried in the tubes did suffer visible deterioration, therefore metals might have become concentrated. The frozen animals may give a more accurate ´picture´ of the situation when the animals were collected. By referring to the results of the fixed range tests it can be seen that none of the preservative solutions differ significantly from the frozen treatment, with the exception of lead at site 1 which has a higher concentration when preserved in formalin.

Whilst it is necessary to be aware that preserving specimens in solutions may affect the metal concentrations within them, there is no evidence that a consistent increase or decrease of metal concentrations occurs.

5.4b Metal concentrations in the decomposers.

5.4b i. Introduction.

The millipede *Glomeris marginata* and the woodlouse *Oniscus asellus* are both macroarthropods which are active in breaking down leaf litter. Both species were found at Haw wood, the most polluted site, therefore it is of interest to examine the metal concentrations in these animals in order to try to understand their method of survival in polluted areas. Further information about *G. marginata* would also supplement the laboratory experiments described in section 5.3d.

Both of these species were common at Haw wood during the period of pitfall trapping, although Hopkin *et al*. (1985) recorded *G. marginata* to be absent in the nearby Hallen wood which is more highly contaminated than Haw wood. Due to the large numbers captured in the present study, some large in size, there seem little doubt that the species is well established at Haw.

5.4b ii. Metal concentrations in *G. marginata*.

All the animals collected in pitfalls in the two weeks prior to 4.7.85 were analysed as described in section 3.1d; each animal was kept separate. The results are given in Table 5.22. The number of animals trapped during the two week period was the same as the number analysed and is given as n. It can be seen that Haw wood was certainly not deficient in animals. The results obtained for dry weight and each metal concentration are compared using oneway analysis of variance. All the tests were significant, so fixed range tests were carried out to compare between means. Due to the unequal sample sizes, the fixed range tests were calculated on the basis of the lowest sample number, therefore the differences shown are conservative estimates. For the metal concentrations, those recorded in animals from Haw are highest in cadmium, copper and zinc, often significantly so. The lead concentrations in animals from the two more polluted woods (Haw and Pegwell) are very similar. The dry weights are variable, with those from Haw wood not significantly different from any of the other woods.

The concentration factors, i.e. the ratio between metal levels in the animals and their food can give an indication of whether metals are accumulated. Table 5.23 shows concentration factors for G. marginata using metal concentrations in the leaf litter from the sites as the values for the food. This will undoubtedly be a simplification because the animals can be more selective in the field, however it should provide a generalised and comparative picture.

Concentration factors were lower at the more polluted sites, which is to be expected. Those for cadmium are all greater

Table 5.22

METAL CONCENTRATIONS ($\mu g g^{-1}$ DRY WEIGHT) IN G. MARGINATA FROM FIVE SITES

SITE	n	DRY WT. (mg)	Cd	Cu	Pb	Zn
WM	23	21.49 ± 3.34^{bc}	15.44 ± 1.68^{b}	45.81 ± 3.53^{ab}	4.81 ± 1.3^{a}	579.3 ± 29.1^{a}
KW	27	9.25 ± 0.94^{a}	3.86 ± 0.56^{a}	32.86 ± 1.74^{a}	11.44 ± 5.43^{ab}	534.9 ± 30.3^{a}
KG	27	28.14 ± 4.27^{c}	4.42 ± 0.38^{a}	47.6 ± 14.8^{ab}	12.43 ± 4.98^{ab}	488.4 ± 19.1^{a}
PW	28	12.24 ± 1.61^{ab}	7.41 ± 0.88^{a}	51.09 ± 2.36^{ab}	32.45 ± 5.19^{c}	564.3 ± 19.0^{a}
HW	66	17.98 ± 1.9^{abc}	26.49 ± 0.74^{c}	71.53 ± 1.1^{b}	30.09 ± 2.91^{bc}	714.8 ± 10.9^{b}
ANOVAR						
F		6.84	151.87	8.52	8.49	26.6
df		4/166	4/166	4/166	4/166	4/166
p		0.01	0.01	0 01	0.01	0.01

Supercripts give the results of a fixed range test, calculated after analysis of variance. Due to the unequal sample sizes the fixed range test was calculated using the smallest value of n. This will result in a conservative estimate of the differences. Means showing the same superscript are not significantly different.

Table 5.23

CONCENTRATION FACTORS BETWEEN G. MARGINATA AND LEAF LITTER AT EACH SITE

	Cd	Cu	Pb	Zn
WM	25.56	3.74	0.15	7.78
KW	3.82	2.4	0.23	4.74
KG	2.16	2.62	0.127	3.06
PW	2.24	2.13	0.23	2.19
HW	1.33	0.47	0.026	0.51

than one, implying that cadmium is accumulated, where as those for lead are all less than one, suggesting that lead is not accumulated. The copper and zinc values tend to be higher at the cleaner sites but less than one at the polluted site. This implies that the animals may be able to regulate these metals, actively uptaking metals at the clean sites and excluding them at Haw. In laboratory studies, juvenile G. marginata accumulated cadmium and zinc but not copper and lead (see section 5.3d). Adults accumulated cadmium, copper and zinc but not lead (Hopkin et al. 1985). It appears that cadmium is accumulated in this species but lead is not. Of the two essential metals, increased concentrations were found in animals from highly polluted areas. However, some degree of regulation may take place which appears to be in the form of maintaining a more or less constant body concentration of zinc. This is seen in Table 5.22 where the Haw animals have zinc concentrations which are significantly higher than the other animals.

G. marginata may live for up to 11 years (Heath et al. 1974), stadial age may be determined up to stadium VI by counting segments but after this it becomes more difficult. Live weight has been used (Heath et al. 1974), although in this study is was not available. As the majority of animals analysed were older than stadium VI, dry weight was used as an indication of age.

At Haw wood 66 individuals were caught and the following remarks apply to this group of animals. Pearson product moment correlation coefficients were calculated for body weight against concentration of each metal in turn. Significant positive results were obtained for cadmium, copper and lead but not zinc ($p<0.05$). This suggests that the first three metals are accumulated with increasing weight (and perhaps age). It is interesting that lead has shown a significant result here, when from evidence given above it was not expected. A possible problem is that the guts of the animals used in this experiment were not voided, nor dissected out. The metals in the guts may constitute a significant part of the total concentrations particularly in animals from Haw. A rough correction for this has indicated that the mean lead concentration in animals from Haw is reduced considerably if the effect of the gut contents is removed; other metals are less affected. This effect may be responsible for the significant increase in lead concentration with increasing weight.

5.4b iii. Metal Concentrations in *O. asellus* variation between sites.

Individuals of *O. asellus* were available from all six sites, trapped over a range of trapping occasions. Five animals from each site were analysed (individually) from four trapping occasions (11.4.85, 4.7.85, 26.9.85, 19.12.85). In addition, where available, five individuals were analysed from sites 1 (Wetmoor) and 6 (Haw wood) for every trap date.

Table 5.24 shows the mean metal concentrations for animals from each site. The first point of interest is that the values are considerably higher than those given in Table 5.22 for _G_. _marginatus_ from the same woods. Two way analysis of variance was calculated for the _O_. _asellus_ data using sites and trapping occasions, with five replicates of each. The F values for the interaction were all significant but small in comparison to that of the main treatments (sites). Table 5.24 gives the results of the F values for comparisons between sites, together with fixed range tests calculated subsequently to compare the means. It can be seen that for cadmium, copper and zinc, concentrations in animals from Haw wood are significantly higher than all the others. For lead, Pegwell wood has animals which have the highest concentrations. It has been shown that woodlice contain high levels of heavy metals when in polluted sites (Martin _et_ _al_. 1976). The majority of metals are stored in the hepatopancreas which may become enlarged because of this (Hopkin & Martin 1982a). Within the hepatopancreas are two types of granules involved in storage (Hopkin & Martin 1982b). Woodlice have also been proposed as useful biological monitors (Hopkin et.al 1986), therefore it is not surprising that differences between the sites were found. It is more of a surprise that lead was not distributed in the same way as other metals.

Table 5.24

MEAN METAL CONCENTRATIONS ($\mu g g^{-1}$) PER SITE FOR O. ASELLUS

SITE	DRY WT. (mg)	Cd	Cu	Pb	Zn
WM	12.85 ± 1.23^{b}	26.06 ± 2.62^{a}	107.12 ± 6.45^{a}	408.5 ± 31.4^{a}	157.0 ± 11.9^{a}
KW	7.98 ± 0.62^{ab}	37.0 ± 2.97^{a}	100.23 ± 6.22^{a}	592.4 ± 35.2^{ab}	172.7 ± 16.9^{a}
KG	10.28 ± 0.65^{ab}	36.59 ± 3.92^{a}	73.3 ± 5.69^{a}	515.6 ± 32.4^{ab}	150.0 ± 6.24^{a}
TP	9.79 ± 0.89^{ab}	46.38 ± 3.13^{a}	96.82 ± 7.6^{a}	627.9 ± 45.5^{ab}	220.7 ± 19.4^{a}
PW	7.81 ± 0.33^{a}	72.57 ± 4.07^{a}	153.28 ± 8.74^{a}	717.7 ± 45.6^{b}	311.4 ± 20.0^{a}
HW	12.06 ± 0.83^{ab}	168.87 ± 8.33^{b}	637.4 ± 36.7^{a}	516.2 ± 38.2^{ab}	696.4 ± 63.2^{b}
F	4.67	106.49	78.17	4.67	36.28
df	5/15	5/15	5/15	5/15	5/15
p	0.05 *	0.01 **	0.01 **	0.05 *	0.01 **

F valves are for treatments (sites) found after two way analysis of variance comparing sites and trapping occasions.

Superscripts give the results of subsequent fixed range tests. Any two means showing the same superscript are not significantly different.

5.4b iv. Metal concentrations in *O. asellus*: variation in time.

Tables 5.25 and 5.26 show the mean metal concentrations of five animals caught at each period of time for Wetmoor and Haw woods. Analysis of variance was carried out on these data, the results of which are also shown. Where significant F values occurred, fixed range tests show that it is usually due to one collection time giving unusually high or low values. The majority of collections appear to be fairly consistent.

O. asellus breeds during the summer and autumn months (Beyer 1957, cited in Warburg *et al*. 1984) and can live for over four years (Collinge 1945). As the dry weights at the different trapping times are not significantly different at either site, it is not possible to identify a cohort of animals throughout a year. It is possible that the period in October and November, where no animals were caught corresponds to the release of young from the brood pouch. These tiny, less active juveniles were not caught in the traps, however, this does not help with the interpretation of the metal concentrations. For Wetmoor there is one trapping occasion for which the animals had higher concentrations of three of the metals. It is probably this collection which is producing a significant F value.

Table 5.25

METAL CONCENTRATIONS ($\mu g g^{-1}$) IN O. ASELLUS FROM SITE 1 (WETMOOR) CAPTURED AT DIFFERENT TIMES OF THE YEAR

TRAPPING OCCASION	DRY WT (mg)	Cd	Cu	Pb	Zn
11.04.85	13.06 ± 2.43	23.38 ± 1.52[a]	98.86 ± 8.25[a]	366.6 ± 54.9[a]	154.2 ± 36.4[a]
25.04	10.71 ± 0.12	22.98 ± 1.09[a]	112.6 ± 7.89[ab]	394.2 ± 5.3[a]	114.9 ± 64.5[a]
09.05	13.87 ± 1.83	30.34 ± 1.55[a]	167.6 ± 13.0[abc]	337.2 ± 42.3[a]	123.4 ± 25.7[a]
23.05	17.01 ± 5.13	23.3 ± 1.28[a]	119.6 ± 7.46[ab]	319.3 ± 52.4[a]	149.3 ± 51.7[a]
06.06	8.73 ± 0.45	73.1 ± 3.99[b]	207.7 ± 14.5[c]	568.2 ± 19.5[ab]	290.9 ± 21.8[b]
20.06	12.77 ± 0.98	20.38 ± 1.98[a]	85.9 ± 7.05[a]	339.9 ± 26.2[a]	129.2 ± 11.8[a]
04.07	15.63 ± 3.12	37.02 ± 8.19[a]	135.6 ± 14.1[ab]	351.8 ± 44.3[a]	198.0 ± 15.2[ab]
01.08	6.6 ± 2.2	24.94 ± 6.81[a]	80.2 ± 18.5[a]	979.0 ± 24.0[b]	187.8 ± 19.9[ab]
29.08	18.22 ± 3.12	31.2 ± 5.41[a]	102.2 ± 8.0[ab]	294.8 ± 43.3[a]	197.2 ± 44.2[ab]
26.09	13.09 ± 2.13	23.66 ± 3.49[a]	86.3 ± 7.59[a]	413.9 ± 82.3[a]	130.0 ± 98.2[a]
10.10	17.64 ± 4.37	27.1 ± 3.85[a]	81.4 ± 20.2[a]	335.7 ± 80.3[a]	116.2 ± 5.3[a]
19.12	9.64 ± 1.93	20.16 ± 2.57[a]	86.0 ± 6.0[a]	501.8 ± 59.3[a]	145.8 ± 19.5[a]
02.01.86	10.67 ± 3.67	33.82 ± 4.34[a]	113.0 ± 24.5[ab]	565.0 ± 111[ab]	190.4 ± 29.8[ab]
F	1.62	11.32	7.82	4.47	3.38
df	12/52	12/52	12/52	12/52	12/52
p	0.05ns	0.05 **	0.01 **	0.01 **	0.01 **

Table 5.26

METAL CONCENTRATIONS ($\mu g g^{-1}$) IN O. ASELLUS FROM SITE 6 (HAW) CAPTURED AT DIFFERENT TIME OF THE YEAR

TRAPPING OCCASION	DRY WT (mg)	Cd	Cu	Pb	Zn
11.04.85	11.6 ± 0.96	116.2 ± 9.95	678.4 ± 32.4^{ab}	555.2 ± 36.4	754.5 ± 36.8^{a}
25.04	12.5 ± 3.2	166.6 ± 9.8	615.1 ± 44.2^{ab}	414.2 ± 40.3	1080.9 ± 79.1^{b}
09.05	12.9 ± 1.4	163.9 ± 14.8	560.4 ± 20.3^{ab}	403.1 ± 12.0	622.0 ± 114^{ab}
23.05	12.6 ± 0.95	190.7 ± 13.8	640.1 ± 23.7^{ab}	484.8 ± 45.1	546.0 ± 41.0^{ab}
06.06	12.12 ± 2.78	167.9 ± 9.3	769.9 ± 30.9^{ac}	465.5 ± 46.9	567.0 ± 55.6^{ab}
20.06	10.39 ± 2.05	163.5 ± 9.8	765.1 ± 23.7^{ac}	495.7 ± 59.0	529.6 ± 30.3^{ab}
04.07	13.2 ± 1.77	194.4 ± 6.6	823.5 ± 48.1^{a}	487.0 ± 9.9	628.7 ± 40.6^{ab}
18.07	12.54 ± 0.88	175.6 ± 9.8	596.0 ± 28.4^{ab}	493.0 ± 44.0	590.4 ± 64.4^{ab}
01.08	11.29 ± 0.94	192.7 ± 25.1	565.9 ± 33.2^{ab}	762.0 ± 167	1098.0 ± 238^{b}
15.08	10.64 ± 2.04	156.4 ± 25.1	561.2 ± 67.2^{ab}	563.7 ± 75.6	501.2 ± 47.6^{a}
29.08	10.52 ± 2.55	173.4 ± 14.6	640.4 ± 20.3^{ab}	498.0 ± 56.2	693.0 ± 97.4^{a}
12.09	8.55 ± 1.57	212.8 ± 31.5	702.4 ± 48.8^{ab}	550.5 ± 64.5	815.0 ± 117^{a}
26.09	9.53 ± 1.56	182.2 ± 24.6	709.6 ± 72.3^{ab}	563.0 ± 124.0	864.0 ± 240.0^{a}
10.10	12.6 ± 1.87	138.2 ± 31.2	571.2 ± 59.5^{ab}	470.8 ± 72.5	642.4 ± 58.4^{b}
21.11	17.8 ± 2.5	188.9 ± 19.8	545.0 ± 51.5^{bc}	513.3 ± 35.3	1527 ± 684.0^{c}
05.12	12.97 ± 2.8	172.6 ± 14.3	563.1 ± 56.0^{ab}	450.0 ± 53.0	641.0 ± 757^{ab}
19.12	13.99 ± 1.93	132.8 ± 5.9	482.2 ± 4.41^{b}	459.9 ± 95.4	538.5 ± 9.89^{ab}
02.01.86	13.7 ± 2.28	205.4 ± 46.5	732.1 ± 120.0^{ab}	553.6 ± 71.4	878.0 ± 159^{a}
16.01	13.07 ± 1.76	156.4 ± 16.8	588.8 ± 71.4^{ab}	541.4 ± 57.3	704.8 ± 66.1^{a}

Table 5.26 contd

TRAPPING OCCASION	DRY WT (mg)	Cd	Cu	Pb	Zn
F	0.95	1.02	3.03	1.18	1.84
df	18/76	18/76	18/76	18/76	18.76
p	0.05ns	0.05ns	0.01 **	0.05ns	0.05

Superscripts give the results of fixed range tests, any two means showing the same superscript are not significantly different.

Table 5.27

PEARSON PRODUCT MOMENT CORRELATION COEFFICIENTS FOR WEIGHT AND METAL CONCENTRATIONS FOR O. ASELLUS FROM TWO SITES

WETMOOR

	Weight	Cd	Cu	Pb
Cd	0.044 ns			
Cu	-0.139ns	0.011ns		
Pb	-0.724*	-0.082ns	0.156ns	
Zn	-0.227*	0.625*	0.209ns	0.154ns

HAW

	Weight	Cd	Cu	Pb
Cd	0.226ns			
Cu	0.177ns	0.508*		
Pb	0.002ns	0.580*	0.199*	
Zn	0.179ns	0.354*	0.019ns	0.339ns

All the animals from each site were lumped together, regardless of time of year of capture and the metal concentrations were correlated with body weight. The results in Table 5.27 also show intercorrelations between metal concentrations. At Wetmoor, lead and zinc were significantly correlated with dry body weight and at Haw wood only cadmium. It seems surprising that more correlations were not significant in animals from Haw wood. However, it has been shown that the variation in speed of growth in woodlice is such that slow growing individuals from one year may be overtaken in size by faster growing individuals of the next. Thus body size is not a good indication of age (Sutton 1972). It is likely that these correlations are not determining whether increase of metal concentrations occurs with age and another method is needed to show this.

5.4c Summary.

1. The effect of different preservatives on metal concentrations in *O. asellus* appears to show no consistent trends.

2. Analysis of metal concentrations in *G. marginata* from 5 sites resulted in significantly different means when tested using analysis of variance.

3. Use of fixed range tests after analysis of variance, calculation of concentration factors and comparison with laboratory tests suggests that cadmium is accumulated in *G. marginata*, lead is not and copper and zinc are accumulated but may also be regulated to some extent.

4. Concentrations of cadmium, copper and lead in *G. marginata* appear to increase as the animals become heavier (this may also be seen as an age effect).

5. Variation in metal concentration in *O. asellus* occurs between differentially polluted sites. Cadmium, copper and zinc were highest from the most polluted site, lead was not so consistent.

6. Variation in metal concentration in *O. asellus* over time from a polluted site and a clean site was significant for some metal but no consistent trends were found.

7. Only cadmium was significantly correlated with body weight of *O. asellus* from the polluted site. It is probable that body size is not a good indication of age so that no variation with age can be shown here.

8. Care must be exercised when comparing concentrations in animals which were analysed complete with their gut contents.

Chapter 6.

THE EFFECT OF HEAVY METAL POLLUTION ON SMALL MAMMALS.

6.1 Introduction.

The subject of metal concentrations in mammals has been approached by many workers, not least because of its implications to human populations. Lead in particular has received much attention due to its release from combustion engines using petrol with lead additives especially in roadside situations. The mammals studied range from mice and voles (e.g. Jefferies & French 1972) to cattle and pigs (Munshower 1977) and humans (Schroeder & Tipton 1968).

The relationship between metals and small mammals has been studied in a variety of situations parallel to those for earthworms (see section 5.1); laboratory trials (Winge *et al*. 1974, Parizek & Zahor 1956); animals monitored from areas receiving applications of sewage sludge contaminated with high levels of metals (Chaney *et al*. 1978, Williams *et al*. 1978); roadside populations (Williamson & Evans 1972, Quarles 1974); animals living on mine spoil heaps (Roberts *et al*. 1978, Andrews *et al*. 1984); and mammals from environments polluted by industrial and/or smelter emissions (Beyer *et al*. 1985, Hunter *et al*. 1987c).

The majority of studies using wild small mammals have been undertaken either in Britain or north America. Whilst most species are not found in both countries, those that are found in America often have parallel species in Britain with similar niches. Therefore, the American studies can be of some use when interpreting results from Britain, although climatic regimes may be different.

Small mammals form links in many food chains and the shrews in particular are important predators on the invertebrate communities. Thus the animals obtained whilst undertaking other studies at the sites afforded an opportunity to examine these animals in more detail.

6.2. Methods.

Usually small mammals are caught using specific methods, either capturing them alive e.g. with a Longworth type trap, or dead using a snap trap. The majority of workers have employed some variation of these two types (e.g. Grainger & Fairley 1978, Bull *et al*. 1977 and Roberts *et al*. 1978). Dry pitfall traps at least 30cm deep containing food can be used (Corbet 1975) however small mammals are also prone to falling into smaller pitfall traps containing preservative fluid intended for invertebrates. This accidental capture has precipitated studies by Butterfield *et al*. (1981) and Yalden (1981), both involving shrews. In the present study mammals were unintentionally captured in the pitfall traps and it is these animals which are discussed below. As well as observation of the numbers of animals at each of the sites, the large numbers available, particularly of the shrews, enabled body metal concentrations to be measured.

Before analysis was performed, the animals were identified and classified as mature or immature using Corbet & Southern (1977) and Yalden (1977). They were then dissected, the guts with their contents removed and the livers and kidneys extracted for separate analysis. The remainder, consisting of the carcass, was analysed as the ´rest´. Digestion and analysis was carried out as described in section 3.1D. Due to the deterioration of specimens before dissection it was difficult to be certain that all the liver had been extracted. Hence calculations testing for differences between the weights of livers have not been attempted.

The numbers of animals analysed for metals do not always correspond to the numbers caught in the pitfall traps. This is because some were considered too decayed for analysis and were discarded. Others were caught outside the ´standard trapping period´ or were from extra traps put down at the sites for other purposes. Statistical tests performed on the metal concentrations used log transformed data (see appendix 1 for details).

6.3. Results and discussion.

6.3a. Pitfall captures.

A total of seven species of mammal were caught. The numbers of each recorded at each site are listed in Table 6.1 Tockington Park caught the most species (6) and Haw the least (2). Haw also caught the smallest number of individuals (11), the largest number being caught at Pegwell wood (95). Spearman rank correlation coefficients between the number of species and the metal concentrations in the leaf litter at each site were all non-significant as were the number of individuals and metal concentrations.

Amongst the extensive literature concerning small mammals and metal concentrations there is very little concerning the distribution of various species in relation to pollution. Only Munshower (1972) has reported an absence of mammals at his most polluted site. It appears from the present data that there is an absence of mammals apart from the genus *Sorex* at Haw. However a similar pattern was found at Wetmoor where, in addition to the shrews, just three individuals of *Clethrionomys glareolus* were caught. The shrews were caught more frequently than the other mammals which suggests that they are present in larger numbers. The larger animals may also be more able to take evasive action when confronted with a trap.

Table 6.1

NUMBERS OF SMALL MAMMALS CAUGHT IN PITFALL TRAPS

AT EACH SITE

SPECIES		HW	PW	TP	KG	KW	WM
Apodemus sylvaticus			6	3	1	2	
Apodemus flavicollis			2				
Clethrionomys glareolus			5	4	1	3	3
Microtus agrestis				5			
Neomys fodiens				1		1	
Sorex araneus	Immature	4	26	38	25	40	6
	Mature	2	5	6	3	7	3
	Total	6	31	44	28	47	9
Sorex minutus	Immature	3	26	11	38	21	5
	Mature	2	25	3	18	10	5
	Total	5	51	14	56	31	10
Number of species		2	5	6	4	5	3
Number of individuals		11	95	71	86	84	22
% of S. minutus as total of Shrews		45.5	62.2	24.2	66.7	39.7	52.6

An interesting feature of these data is the relatively high proportion of *Sorex minutus* over *Sorex araneus* at several of the sites. It has been noted several times that *S. minutus* is less common than *S. araneus*. Corbet & Southern (1977) list that only 10-16% of shrews in grassland are *S. minutus* and only 4% in woodlands. Both Ellenbroek (1980) and Crowcroft (1957) list *S. minutus* as scarce in woods. The only exceptions to this relative scarcity appear to be in sand dunes (Grainger & Fairley 1978) on moorland (Butterfield *et al*. 1981, Yalden 1981) and in Ireland where *S. araneus* does not occur (Corbet & Southern (1977). Yalden (1981) gives a table summarising the percentage of *S. minutus* in samples from other authors. The highest percentage excluding his moorland data is 46.9 in Dutch dunes, the highest for woodlands is 20.6. By referring to Table 6.1, the percentage of *S. minutus* at the six sites sampled in the present study is shown. The lowest figure is 24.2 for Tockington Park and the highest (66.7 for Kington Grove) is far higher than reported in any other woodland study. Yalden (1981) and Butterfield *et al*. (1981) concluded that the lower numbers of *S. araneus* in dunes and moorland is due to lack of food. This species feeds largely on earthworms (discussed more fully in section 6.3c.iv) which are reduced in numbers in moorland and duneland habitats. Although number of worms were very low at Kington Grove (section 5.1d) it seems remarkable that all the sites used show larger numbers of *S. minutus* than expected from the literature. Yalden (1981) also concluded that the traps used by him were not biased in favour of S. minutus because when trapping at a lowland site more S. araneus were caught. However, as with the invertebrate captures it must be remembered that the number of animals pitfall trapped is a function of both abundance and activity. S. minutus is faster and more active than S. araneus (Corbet & Southern 1977) and spends more time foraging on the surface of the litter. S. araneus is a strong digger (Pernetta 1977) a successful subterranean forager (Churchfield 1980) and in winter may spend 80% of its time below ground (Michielsen 1966). Accordingly, S. minutus seems more likely to encounter pitfall traps. It is perhaps significant that both of the moorland studies (Butterfield et al. 1981 and Yalden 1981) and the Dutch dune survey (Michielsen 1966) caught animals by pitfall trapping. Despite this, the percentage of S. minutus at some of the sites in the present study is remarkably higher than any other woodland studies and is worthy of note.

6.3b. Metal concentrations in small mammals (excepting the genus Sorex).

The metal concentrations in five species of mammals caught (ie. excluding S. minutus and S. araneus) are given in Tables 6.2, 6.3, 6.4. Microtus agrestis and Apodemus flavicolis were recorded from only one site each and Neomys fodiens, although from three sites, has only one replicate

Table 6.2

METAL CONCENTRATIONS ($\mu g g^{-1}$ DRY WEIGHT) IN THE 'REST OF BODY' COMPONENT OF SMALL MAMMALS

EXCLUDING THE SHREWS.

SPECIES	SITE	n	Cd	Cu	Pb	Zn
Clethrionomys glareolus	WM	3	0.7 ± 0.54	11.23 ± 1.7	2.99 ± 0.43	103.9 ± 13.7
	KW	3	0.67 ± 0.51	11.75 ± 0.56	4.21 ± 1.25	109.35 ± 4.62
	TP	4	0.38 ± 0.28	9.09 ± 0.61	3.84 ± 1.11	96.26 ± 3.61
	PW	4	0.99 ± 0.21	13.8 ± 0.84	6.89 ± 1.53	112.45 ± 5.08
ANOVAR F		df 3/10	0.53 ns	3.63 ns 2	1.97 ns	1.13 ns
Apodemus sylvaticus	KW	4	3.2 ± 1.72	11.5 ± 1.73	16.98 ± 7.97	114.9 ± 13.3
	KG	1	0.43	10.62	1.53	113.4
	TP	2	0.99 ± 0.06	11.99 ± 0.97	3.74 ± 2.24	106.76 ± 4.76
	PW	5	0.51 ± 0.1	11.88 ± 0.46	7.69 ± 0.54	94.15 ± 2.26
ANOVAR F (-site KG)		df 2/8	1.91 ns	0.04 ns	1.54 ns	1.73 ns
Microtus agrestis	TP	6	0.69 ± 0.13	16.47 ± 0.91	5.4 ± 0.96	101.86 ± 7.96
Apodemus flavicollis	PW	2	1.62 ± 1.25	17.85 ± 3.4	11.78 ± 4.3	101.8 ± 15.1
Neomys fodiens	KW	1	0.24	16.94	4.34	114.23
	KG	1	0.096	14.57	5.79	117.12
	TP	1	0.43	20.68	8.84	112.02

Table 6.3

METAL CONCENTRATIONS IN ($\mu g g^{-1}$) MAMMAL LIVERS (EXCLUDING SHREWS)

SPECIES	SITE	n	Cd	Cu	Pb	Zn
Clethrionomys glareolus	WM	3	1.3 ± 0.18	17.52 ± 4.55	0.8 ± 0.38	75.3 ± 10.7
	KW	3	0.75 ± 0.51	21.89 ± 3.35	0.84 ± 0.31	74.5 ± 5.9
	TP	3	1.81 ± 1.55	11.14 ± 1.63	1.46 ± 0.59	102.6 ± 20.9
	PW	5	0.82 ± 0.47	13.61 ± 1.25	3.16 ± 1.39	76.2 ± 12.0
Anovar F		$df_{3/10}$	0.36 ns	2.92 ns	1.24 ns	0.90 ns
Apodemus sylvaticus	KW	2	1.39 ± 0.76	14.6 ± 1.35	0.63 ± 0.03	86.94 ± 9.5
	KG	1	1.462	12.685	1.163	91.438
	TP	2	2.26 ± 0.26	11.35 ± 3.36	3.12 ± 2.62	78.6 ± 0.01
	PW	6	0.65 ± 0.22	18.9 ± 3.9	2.22 ± 0.52	50.5 ± 9.83
Anovar F		$df_{2/7}$	5.29 $p<0.05$	0.68 ns	1.01 ns	2.91 ns
Microtus agrestis	TP	5	1.62 ± 0.87	16.53 ± 5.25	0.66 ± 0.59	68.6 ± 13.5
Apodemus flavicolis	PW	2	1.82 ± 1.17	21.19 ± 2.5	3.66 ± 1.5	66.83 ± 7.56
Neomys fodiens	KW	1	0.548	24.768	0.713	94.78
	TP	1	0.689	53.45	4.71	62.07

Table 6.4

METAL CONCENTRATIONS IN ($\mu g g^{-1}$) MAMMAL KIDNEYS (EXCLUDING SHREWS)

SPECIES	SITE	n	Cd	Cu	Pb	Zn
Clethrionmys glareolus	WM	3	1.65 ± 0.478	4.01 ± 2.01	0.713 ± 0.71	77.14 ± 7.99
	KW	3	0.55 ± 0.174	8.14 ± 2.74	2.15 ± 0.97	68.62 ± 9.44
	TP	2	2.14 ± 1.82	8.73 ± 0.79	2.38 ± 1.33	43.1 ± 22.6
	PW	5	1.18 ± 0.65	7.59 ± 0.92	2.13 ± 1.22	33.6 ± 13.3
Anovar F		$df_{3/9}$	0.63 ns	1.32 ns	0.37 ns	2.48 ns
Apodemus sylvaticus	KW	2	2.34 ± 0.97	1.73 ± 0.83	0.81 ± 0.11	83.83 ± 8.04
	KG	1	4.394	9.091	2.97	95.45
	TP	2	2.87 ± 0.6	8.23 ± 0.38	2.19 ± 2.18	60.2 ± 15.5
	PW	5	1.27 ± 0.602	2.38 ± 1.16	8.06 ± 2.07	50.0 ± 16.4
Anovar F		$df_{2/6}$	1.38 ns	5.99 $p<0.05$	3.61 ns	0.81 ns
Microtus agrestis	TP	5	2.36 ± 0.94	8.29 ± 2.68	1.29 ± 1.16	62.7 ± 17.2
Apodemus flavicolis	PW	2	2.32 ± 0.65	10.07 ± 2.67	5.85 ± 3.75	48.1 ± 18.1
Neomys fodiens	KW	1	0.592	9.605	1.424	46.71
	TP	1	0.926	4.629	18.935	92.59

for each, hence no comparisons between sites can be made for these species. Analysis of variance for Apodemus sylvaticus from three sites where more than one individual was caught shows significant differences between the sites for concentrations of cadmium in the livers and copper in the kidneys. Because of the low number of replicates not too much emphasis can be placed on these results. Clethrionomys glareolus was caught from four sites, analysis of variance for the four metals and three body compartments were all non-significant.

The results indicate that there is little difference between the sites for these species. Although none of these species were caught at Haw, there does not appear to be an increase in heavy metal concentrations at Pegwell wood, the second highest polluted site sampled in.

Laboratory experiments have shown that cadmium and lead are accumulated in small mammal tissues in proportion to the dosage injected (Winge et al. 1974) or fed in the diet (Chaney et al. 1978, Mierau et al. 1975). In wild populations, the lead concentrations in small mammals has been shown to increase in those living close to busy roads (Goldsmith & Scanlon 1977, Welch & Dick 1975). Lead and zinc concentrations were found to be significantly higher in animals from mine sites in comparison to controls (Roberts & Johnson 1978). Cadmium and copper concentrations were also highest in species found close to a copper refinery (Hunter *et al*. 1987c).

6.3c. Metal concentrations in the two *Sorex* species.

6.3ci. Metal concentrations and sites.

S. araneus and *S. minutus* were caught at all six sites, sometimes in large numbers. Both immatures and matures occurred although the matures were always out numbered. Table 6.5 shows the mean metal concentrations for immatures and matures of *S. araneus* for each site. Results of analysis of variance are shown together with the results of t-tests undertaken after the analysis of variance to provide comparisons between the sample means. Tables 6.6 and 6.7 consider the livers and kidneys of this species, whilst Tables 6.8, 6.9 and 6.10 give similar information for *S. minutus*.

In the majority of instances significant differences in metal concentrations between the sites are demonstrated. With reference to the results of the subsequent t-tests it can be seen that it is usually animals from Haw which have considerably higher concentrations than the other sites. Pegwell is also often higher than the other cleaner sites. This is particularly true for cadmium. Table 6.11 shows the total body concentrations of the four metals in the shrews

Table 6.5

METAL CONCENTRATIONS ($\mu g g^{-1}$ DRY WEIGHT) IN 'REST OF BODY' OF S. ARANEUS

SITE	IMMATURES					MATURES				
	Wt	Cd	Cu	Pb	Zn	Wt	Cd	Cu	Pb	Zn
WM	1.701 ±0.087	2.052^{abc} ±0.872	11.86^{a} ±0.972	4.671^{a} ±0.714	117.46^{a} ±3.28	2.516 ±0.386	4.58^{a} ±1.01	13.12^{a} ±1.36	6.8^{a} ±2.19	133.07 ±5.99
n	8	8	8	8	8	3	3	3	3	3
KW	1.635 ±0.042	1.628^{a} ±0.266	12.253^{a} ±0.21	6.477^{a} ±0.422	127.57^{b} ±2.1	2.43 ±0.204	5.272^{a} ±0.817	12.51^{a} ±1.94	7.62^{a} ±1.95	141.3^{ab} ±4.57
n	39	39	39	39	39	6	6	6	6	6
KG	1.557 ±0.051	2.606^{b} ±0.439	13.67^{b} ±0.57	10.48^{b} ±0.97	128.76^{ab} ±3.9	2.53 ±0.311	3.58^{ab} ±1.19	11.183^{a} ±0.526	2.751^{a} ±0.801	131.57^{a} ±5.02
n	33	33	33	33	33	3	3	3	3	3
TP	1.583 ±0.048	4.51^{cd} ±0.99	13.638^{ab} ±0.433	17.26^{c} ±1.58	147.62^{c} ±4.91	2.529 ±0.207	8.65^{b} ±1.09	14.762^{b} ±0.754	20.74^{b} ±4.37	154.12^{cd} ±2.92
n	29	29	29	29	29	9	9	9	9	9
PW	1.601 ±0.0496	4.441^{d} ±0.808	14.027^{ab} ±0.731	20.64^{c} ±0.96	140.6^{bc} ±5.99	2.805 ±0.239	233.3^{c} ±36.9	15.37^{bc} ±1.35	34.86^{c} ±2.79	163.8^{acd} ±18.3
n	24	24	24	24	24	5	5	5	5	5
HW	1.352 ±0.119	77.6^{c} ±67.2	21.22^{c} ±1.93	66.6^{d} ±14.5	185.0^{c} ±20.5	2.436 ±0.281	326.3^{c} ±47.0	21.71^{cd} ±2.44	89.8^{d} ±19.9	183.5^{bd} ±15.0
n	7	7	7	7	7	4	4	4	4	4
f	1.72	13.77	8.08	43.82	7.52	0.26	107.6	2.4	16.47	3.52
p	0.05ns	0.01**	0.01**	0.01**	0.01**	0.05ns	0.01**	0.05ns	0.01**	0.05*

Table 6.6

METAL CONCENTRATIONS ($\mu g g^{-1}$ DRY WEIGHT) IN LIVERS OF S. ARANEUS

SITE	IMMATURE					MATURE				
	Wt (g)	Cd	Cu	Pb	Zn	Wt (g)	Cd	Cu	Pb	Zn
WM	0.118 $\pm$0.038	7.37^{a} $\pm$5.27	14.82^{bc} $\pm$2.86	1.736^{ab} $\pm$0.96	84.1^{ac} $\pm$15.0	0.129 $\pm$0.016	14.36^{ab} $\pm$0.76	22.9^{a} $\pm$5.03	0.48^{a} $\pm$0.25	96.74^{a} $\pm$8.63
n	7	7	7	7	7	3	3	3	3	3
KW	0.099 $\pm$0.022	4.3^{a} $\pm$0.817	11.58^{ab} $\pm$0.929	1.513^{a} $\pm$0.452	75.25^{a} $\pm$4.96	0.082 $\pm$0.011	46.2^{c} $\pm$12.9	23.24^{a} $\pm$2.79	2.05^{ac} $\pm$0.64	126.8^{ab} $\pm$14.0
n	37	37	37	37	37	6	6	6	6	6
KG	0.056 $\pm$0.006	11.87^{b} $\pm$5.22	23.25^{cd} $\pm$7.4	8.65^{b} $\pm$5.89	249^{cd} $\pm$142	0.329 $\pm$0.23	14.9^{a} $\pm$12.6	11.19^{a} $\pm$3.95	0.49^{a} $\pm$0.34	54.3^{ab} $\pm$20.7
n	19	19	19	19	19	3	3	3	3	3
TP	0.0762 $\pm$0.006	13.49^{b} $\pm$2.63	14.67^{bd} $\pm$1.26	3.927^{b} $\pm$0.655	139.9^{bc} $\pm$34.6	0.102 $\pm$0.024	68.0^{c} $\pm$19.2	20.85^{a} $\pm$1.71	5.57^{b} $\pm$1.09	142.5^{b} $\pm$14.4
n	30	30	30	30	30	10	10	10	10	10
PW	0.105 $\pm$0.028	9.11^{b} $\pm$2.04	9.57^{a} $\pm$1.10	2.752^{b} $\pm$0.567	73.3^{a} $\pm$6.99	0.134 $\pm$0.026	164.0^{d} $\pm$53.0	28.75^{a} $\pm$5.3	5.14^{bc} $\pm$1.28	145.3^{b} $\pm$16.6
n	19	19	19	19	19	5	4	5	5	5
HW	0.065 $\pm$0.012	111.8^{c} $\pm$45.0	32.5^{e} $\pm$5.65	15.32^{c} $\pm$4.61	158.3^{d} $\pm$17.5	0.122 $\pm$0.022	452^{bcd} $\pm$407	23.32^{a} $\pm$1.39	41.8^{a} $\pm$30.6	196.5^{ab} $\pm$59.6
n	9	9	9	9	9	4	2	2	2	2

Table 6.6 contd

SITE	IMMATURE					MATURE				
	Wt (g)	Cd	Cu	Pb	Zn	Wt (g)	Cd	Cu	Pb	Zn
f	+	13.9	5.66	8.76	4.57	+	3.81	2.85	10.14	4.8
p		0.01**	0.01**	0.01**	0.01**		0.01**	0.05*	0.01**	0.01**

+ No statistical tests carried out, due to the problems of ensuring *all* the liver was extracted from the carcasses.

Table 6.7

METAL CONCENTRATIONS ($\mu g g^{-1}$ DRY WEIGHT) IN KIDNEYS OF S. ARANEUS

SITE	IMMATURES					MATURES				
	Wt	Cd	Cu	Pb	Zn	Wt	Cd	Cu	Pb	Zn
WM	0.0183 $\pm$0.0016	6.67^{abc} $\pm$4.44	6.52^{ab} $\pm$2.07	2.217^{ab} $\pm$0.345	58.56^{bc} $\pm$8.91	0.069 $\pm$0.0021	10.88^{a} $\pm$1.17	8.802 $\pm$0.984	1.804^{a} $\pm$0.952	76.96^{a} $\pm$3.31
n	6	7	7	7	7	3	3	3	3	3
KW	0.0198 $\pm$0.002	3.616^{a} $\pm$0.689	6.732^{ab} $\pm$0.991	2.95^{a} $\pm$0.353	54.01^{ab} $\pm$5.83	0.0177 $\pm$0.0021	37.0^{a} $\pm$15.3	9.79 $\pm$2.41	9.67^{ac} $\pm$2.33	137.4^{ab} $\pm$34.3
n	36	36	36	36	36	6	6	6	6	6
KG	0.0348 $\pm$0.0215	7.66^{b} $\pm$3.08	10.92^{b} $\pm$1.89	5.239^{abcd} $\pm$0.827	108.4^{c} $\pm$10.2	0.028 $\pm$0.007	9.28^{a} $\pm$4.92	10.83 $\pm$4.84	1.912^{a} $\pm$0.675	55.1^{ab} $\pm$25.7
n	19	19	19	19	19	3	3	3	3	3
TP	0.0152 $\pm$0.00196	19.81^{cd} $\pm$5.58	6.05^{ac} $\pm$1.22	13.26^{d} $\pm$3.6	155.8^{c} $\pm$38.2	0.019 $\pm$0.0032	41.3^{a} $\pm$12.7	7.76 $\pm$1.86	19.26^{bc} $\pm$3.79	122.9^{b} $\pm$15.5
n	30	30	30	30	30	30	10	10	10	10
PW	0.029 $\pm$0.0058	10.28^{bd} $\pm$2.8	2.959^{a} $\pm$0.534	5.49^{bc} $\pm$1.11	47.2^{a} $\pm$10.8	0.0229 $\pm$0.0045	154.2^{ac} $\pm$37.0	13.91 $\pm$2.59	20.08^{b} $\pm$3.68	109.33^{b} $\pm$8.17
n	19	19	19	19	19	5	5	5	5	5
HW	0.0474 $\pm$0.0336	86.7^{e} $\pm$37.8	19.27^{bc} $\pm$4.44	38.7^{e} $\pm$13.6	143.0^{c} $\pm$27.9	0.0216 $\pm$0.0048	142.3^{bc} $\pm$98.7	13.65 $\pm$4.27	380^{ab} $\pm$358	200.9^{ab} $\pm$81.1
n	9	9	9	9	9	2	2	2	2	2
f	1.81	8.00	2.4	6.7	5.61	1.01	4.34	0.81	9.62	2.65
p	0.05ns	0.01**	0.05*	0.01**	0.01**	0.05ns	0.01**	0.05ns	0.01**	0.05*

Table 6.8

METAL CONCENTRATIONS ($\mu g g^{-1}$ DRY WEIGHT) IN 'REST OF BODY' OF S. MINUTUS

SITE	IMMATURES					MATURES				
	Wt	Cd	Cu	Pb	Zn	Wt	Cd	Cu	Pb	Zn
WM	0.7075^{a} ± 0.019	2.309^{cd} ± 0.283	14.120^{bc} ± 0.048	7.856^{a} ± 0.703	131.81^{b} ± 6.21	1.282^{a} ± 0.117	3.774^{ac} ± 0.717	14.12^{ac} ± 1.48	8.305^{a} ± 0.615	104.12^{a} ± 4.55
n	13	13	13	13	13	6	6	6	6	6
KW	0.7653^{ab} ± 0.029	1.015^{a} ± 0.305	12.313^{a} ± 0.45	8.414^{a} ± 0.655	115.31^{a} ± 3.46	1.171^{abd} ± 0.109	2.065^{a} ± 0.243	13.113^{a} ± 0.613	8.738^{a} ± 0.534	118.77^{b} ± 2.36
n	18	18	18	18	18	15	15	15	15	15
KG	0.712^{a} ± 0.0124	1.717^{b} ± 0.269	15.323^{bc} ± 0.681	11.059^{b} ± 0.608	125.11^{bd} ± 2.71	0.956^{ab} ± 0.0719	3.229^{a} ± 0.415	15.336^{c} ± 0.822	13.502^{b} ± 0.958	124.55^{bd} ± 4.2
n	43	43	43	43	43	18	18	18	18	18
TP	0.895^{b} ± 0.963	2.896^{bc} ± 0.801	13.79^{b} ± 0.516	15.66^{c} ± 1.67	128.77^{b} ± 3.62	1.148^{acd} ± 0.630	5.521^{bcd} ± 0.488	15.999^{c} ± 0.417	21.17^{c} ± 3.14	123.25^{be} ± 5.38
n	18	18	18	18	18	18	7	7	7	7
PW	0.745^{ab} ± 0.0153	4.315^{c} ± 0.588	15.363^{c} ± 0.43	28.48^{d} ± 1.46	134.89^{e} ± 3.32	1.184^{acd} ± 0.336	7.998^{b} ± 0.45	16.512^{c} ± 0.565	31.86^{d} ± 1.56	136.68^{cde} ± 4.52
n	27	27	27	27	27	25	25	25	25	25
HW	0.709^{ab} ± 0.094	4.29^{cde} ± 1.13	20.1^{abc} ± 4.32	91.7^{e} ± 10.3	150.5^{cd} ± 13.9	0.93^{acd} ± 0.173	12.00^{bd} ± 3.59	20.911^{d} ± 0.371	102.42^{e} ± 4.07	191.1^{c} ± 22.2
n	6	6	6	6	6	4	4	4	4	4
f	2.89	11.65	4.69	82.58	4.04	3.16	21.85	6.27	94.31	9.67
p	0.05*	0.01**	0.01**	0.01**	0.01**	0.05*	0.01**	0.01**	0.01**	0.01**

Table 6.9

METAL CONCENTRATIONS ($\mu g g^{-1}$ DRY WEIGHT IN LIVERS OF S. MINUTUS

SITE	IMMATURE					MATURE				
	Wt (g)	Cd	Cu	Pb	Zn	Wt (g)	Cd	Cu	Pb	Zn
WM	0.284 $\pm$0.004	5.147^{ad} $\pm$0.863	12.45^{bc} $\pm$1.66	1.3 $\pm$0.201	110.0 $\pm$11.8	0.568 $\pm$0.009	13.15^{ac} $\pm$2.82	15.04 $\pm$2.86	0.87^{a} $\pm$0.197	87.35 $\pm$5.9
n	11	11	11	11	11	6	6	6	6	6
KW	0.038 $\pm$0.008	2.224^{a} $\pm$0.429	11.61^{b} $\pm$1.73	2.658 $\pm$0.827	62.54^{a} $\pm$7.91	0.041 $\pm$0.009	7.37^{a} $\pm$1.5	14.5 $\pm$2.89	1.846^{ac} $\pm$0.347	96.03 $\pm$8.4
n	18	18	18	18	18	7	7	7	7	7
KG	0.298 $\pm$0.0028	3.25^{ac} $\pm$0.584	25.52^{cd} $\pm$6.09	3.239 $\pm$0.819	136.4^{bc} $\pm$51.8	0.046 $\pm$0.007	10.72^{a} $\pm$3.01	18.74 $\pm$3.61	2.6^{bc} $\pm$0.504	87.76 $\pm$8.38
n	30	30	30	30	30	14	14	14	14	14
TP	0.038 $\pm$0.007	7.36^{bcd} $\pm$1.76	15.21^{bd} $\pm$1.91	2.75 $\pm$0.598	100.4^{c} $\pm$11.1	0.077 $\pm$0.015	21.7^{bc} $\pm$2.65	22.7 $\pm$1.41	3.114^{c} $\pm$0.563	115.5 $\pm$12.3
n	14	14	14	14	14	7	7	7	7	7
PW	0.043 $\pm$0.005	12.58^{bd} $\pm$2.45	7.51^{a} $\pm$1.1	5.44 $\pm$1.79	85.2 $\pm$16.3	0.052 $\pm$0.005	29.57 $\pm$3.41	14.89^{b} $\pm$1.7	4.186^{c} $\pm$0.645	99.67 $\pm$7.42
n	18	18	18	18	18	27	27	27	27	27
HW	0.043 $\pm$0.014	25.03^{b} $\pm$8.47	31.8^{bd} $\pm$13.0	6.26 $\pm$2.01	94.27^{c} $\pm$9.31	0.047 $\pm$0.008	63.7^{c} $\pm$23.3	19.46 $\pm$3.35	13.98^{d} $\pm$5.23	120.7 $\pm$12.4
n	5	5	5	5	4	4	4	4	4	4

Table 6.9 contd

SITE	IMMATURE					MATURE				
	Wt (g)	Cd	Cu	Pb	Zn	Wt (g)	Cd	Cu	Pb	Zn
F	+	7.25	5.16	2.14	2.81	+	6.27	1.00	5.14	1.08
df		5/90	5/09	5/90	5/90		5/59	5/59	5/59	5/59
p		0.01**	0.01**	0.05ns	0.05*		0.01**	0.05ns	0.01**	0.05ns

+ No statistical tests carried out, due to the problems of ensuring all the liver was extracted from the carcasses.

Table 6.10

METAL CONCENTRATIONS ($\mu g g^{-1}$ DRY WEIGHT) IN KIDNEYS OF S. MINUTUS

SITE	IMMATURES					MATURES				
	Wt	Cd	Cu	Pb	Zn	Wt	Cd	Cu	Pb	Zn
WM	0.0099^{a} ± 0.00175	5.471^{bd} ± 0.851	9.12^{bd} ± 2.07	2.33^{a} ± 1.15	135.7^{d} ± 27.5	0.0144 ± 0.0014	7.91^{ac} ± 1.61	9.56^{abc} ± 5.69	42.0 ± 40.5	77.9 ± 20.4
n	11	11	11	11	11	6	6	6	6	6
KW	0.0105^{ab} ± 0.0013	1.986^{a} ± 0.515	5.76^{ac} ± 1.46	2.577^{ab} ± 0.501	29.56^{ab} ± 8.01	0.0132 ± 0.00164	5.92^{a} ± 1.47	4.77^{ab} ± 1.43	2.046 ± 0.581	71.5 ± 16.6
n	17	17	17	17	17	8	8	8	8	8
KG	0.0092^{a} ± 0.0014	2.46^{a} ± 0.458	17.8^{bd} ± 2.98	5.253^{b} ± 0.85	97.6^{bc} ± 12.4	0.0111 ± 0.0023	7.95^{a} ± 2.5	16.78^{bd} ± 5.79	4.904 ± 0.977	109.0 ± 17.6
n	32	32	32	32	32	11	11	11	11	11
TP	0.0095^{b} ± 0.0011	4.15^{ac} ± 1.07	13.0^{bc} ± 3.95	3.777^{b} ± 0.553	101.6^{cd} ± 11.7	0.0127 ± 0.0014	12.142^{bc} ± 0.926	3.77^{a} ± 2.69	3.866 ± 0.986	94.87 ± 9.69
n	14	14	14	14	14	6	6	6	6	6
PW	0.0565^{ab} ± 0.043	9.27^{bcd} ± 1.92	2.87^{a} ± 1.15	5.53^{b} ± 1.61	66.8^{b} ± 16.1	0.0165 ± 0.002	18.76^{c} ± 2.46	4.95^{a} ± 1.46	6.75 ± 1.02	73.79 ± 9.49
n	18	18	18	18	18	27	27	27	27	27
HW	0.0138^{ab} ± 0.00197	15.81^{d} ± 5.92	18.2^{acd} ± 14.2	12.39^{c} ± 3.52	78.2^{bcd} ± 12.9	0.0156 ± 0.0046	49.9^{c} ± 19.3	14.03^{cd} ± 4.54	51.0 ± 25.9	152.8 ± 52.8
n	5	5	5	5	5	4	4	4	4	4
f	1.98	4.06	4.27	3.98	6.97	1.29	4.07	2.79	1.76	0.70
df	5/91	5/91	5/91	5/90	5/91	5/56	5/56	5/56	5/56	5/56
p	0.05ns	0.01**	0.01**	0.01**	0.01**	0.01ns	0.01**	0.05*	0.05ns	0.05ns

Table 6.11

TOTAL BODY CONCENTRATIONS ($\mu g g^{-1}$ DRY WEIGHT) OF THE FOUR METALS IN THE SHREWS (CALCULATED FROM MEANS)

	SITE	Cd	Cu	Pb	Zn
S. araneus					
Immature	WM	2.44	12.54	4.46	114.74
	KW	1.93	12.12	5.9	120.92
	KG	3.03	13.93	10.3	132.41
	TP	5.05	13.61	16.62	147.34
	PW	9.78	13.57	19.3	134.96
	HW	79.41	21.66	63.43	182.47
Mature	WM	5.20	13.47	6.37	129.92
	KW	6.83	12.84	7.06	140.8
	KG	4.92	11.18	2.48	117.46
	TP	10.92	14.61	19.69	149.99
	PW	229.55	15.96	33.4	162.54
	HW	330.67	21.71	90.0	184.26
S. minutus					
Immature	WM	2.46	13.99	7.54	131.08
	KW	1.08	12.2	8.07	111.81
	KG	1.79	15.75	10.68	125.19
	TP	3.09	13.84	15.02	127.36
	PW	5.06	14.13	25.53	127.84
	HW	5.66	20.72	85.45	146.02
Mature	WM	4.21	14.11	9.21	103.14
	KW	2.28	13.07	8.44	117.51
	KG	3.62	15.51	12.91	122.7
	TP	6.66	16.22	19.86	122.47
	PW	9.03	16.3	30.39	134.34
	HW	15.06	20.73	97.41	187.15

(calculated from means). It appears that all the metals are probably accumulated in the shrews in particular cadmium and lead. The data for the shrews is more in line with the literature on metal concentrations than the other small mammals in this study.

6.3c ii. Metal concentrations in the different body compartments.

It has been demonstrated many times that lead levels in mammals are highest in the bones (e.g. Smith *et al* 1970, Getz *et al*. 1977 and Schroeder & Tipton 1968 who showed that 91% of total body lead in humans is in the bones). Zinc may also be accumulated in bone (Johnson *et al*. 1978). In contrast, cadmium is found at highest concentrations in the kidney and liver (Andrews *et al*. 1984, and Hunter & Johnson 1982 who recorded more than 80% of the body cadmium to be in the kidney and liver). These high concentrations can cause damage to the tissues (Hunter *et al*. 1984b). Sawicka-Kapusta & Kozlowski (1984) have shown that little cadmium occurs in the hair of small mammals (although a large proportion in birds is found in the feathers).

Table 6.12 shows the ordering of body compartments according to metal concentrations for the shrews from Haw. Whilst on the whole the highest concentrations of lead and zinc are found in the ′rest′ component (ie. the compartment which included the bones), cadmium in the liver and kidney and copper in the liver, complete agreement with the literature is not found.

Table 6.13 presents the percentage of the total metals found in the kidney (calculated from tables using means). The results for cadmium are many times lower than would be expected according to Hunter & Johnson (1982). Andrews et al. (1984) indicated that although the kidneys may be the target organ for cadmium, a shift to accumulation in the liver may occur when the kidneys become damaged by the pollution; this has also been suggested by Hunter et al. (1981). In the study by Andrews et al. (1984) S. araneus at mine sites had mean concentrations of cadmium ($\mu g\ g^{-1}$ dry weight) in the liver, kidney and rest of body of 235.8, 158.1 and 52.7 respectively. In the present study the same species at Haw had concentrations of 452.0, 142.3 and 326.3 in the same body components. The high concentrations in the ´rest´ component at Haw suggests that another organ may also be important in accumulation of cadmium. Alternatively, because extracting all the liver was difficult, much may have been left in the carcass.

6.3c iii. Variation of metal concentrations (and weight) with age.

Increased metal concentration with age has been recorded for lead, in humans (Schroeder & Tipton 1968), cattle & pigs (Munshower 1977) and wild small mammals (Quarles 1974,

Table 6.12

THE ORDERING OF CONCENTRATIONS OF METALS IN EACH BODY COMPONENT. (All animals from Haw)

Based on populations means

Species/Maturity	Cd	Cu	Pb	Zn
S. minutus Imm	L>K>R	L>R>K	R>K>L	R>L>K
Mat	L>K>R	R>L>K	R>L>K	R>K>L
S. araneus Imm	R>K>L	L>R>K	R>K>L	R>L>K
Mat	L>R>K	L>R>K	K>R>L	K>L>R

Key: L = Liver K = Kidney R = Rest of Body

Table 6.13

PERCENTAGE METAL IN THE KIDNEY FOR THE SHREWS AT EACH SITE

	SITE	Wt % Kidney	Cd % Kidney	Cu % Kidney	Pb % Kidney	Zn % Kidney
S. araneus Immature	WM	0.98	2.68	4.86	0.49	0.5
	KW	1.08	1.95	0.58	0.54	0.3
	KG	2.12	5.41	1.65	1.08	1.7
	TP	0.89	3.51	0.39	0.71	0.95
	PW	1.67	1.76	0.37	0.47	0.58
	HW	3.21	3.5	2.87	1.96	2.52
Mature	WM	2.54	5.31	1.67	0.72	1.51
	KW	0.71	3.88	0.54	0.97	0.69
	KG	0.97	1.83	0.93	0.74	0.45
	TP	0.7	2.65	0.38	0.69	0.57
	PW	0.78	0.52	0.68	0.47	0.52
	HW	0.86	0.37	0.54	3.6	0.93
S. minutus Immature	WM	1.33	2.95	0.86	0.41	1.37
	KW	1.29	2.38	0.6	0.41	0.34
	KG	1.2	1.64	1.35	0.59	0.93
	TP	0.95	1.27	0.9	0.24	0.76
	PW	6.63	12.14	1.35	1.43	3.47
	HW	1.83	5.09	1.61	0.26	0.98
Mature	WM	1.03	1.95	0.7	5.21	0.78
	KW	1.06	2.75	0.39	0.25	1.06
	KG	1.09	2.37	1.18	0.41	0.65
	TP	1.05	1.92	0.24	0.2	0.81
	PW	1.28	2.65	0.39	0.28	0.7
	HW	1.61	5.34	1.09	0.84	1.32

Schlesinger & Potter 1974, Munshower 1972). Cadmium concentration was found to increase with age in an American mouse species (Peromyscus leucopus) but not in the vole Microtus pennsylvanicus (Smith & Rongstad 1982). Hunter et al. (1981) have shown that within juvenile S. araneus cadmium concentrations were positively correlated with age (measured as body weight). Zinc and copper concentrations however may not increase with age (Williams et al. 1978).

Shrews can be relatively easily classified as immature or mature and Tables 6.14 and 6.15 show the results of t-tests, comparing the two age classes in each species for the 'rest' component (mean values are repeated for added clarity). The results show that the concentrations of cadmium increase with age in both species except at two of the clean sites. The other metals do not show such a clear picture. At most sites there is no difference between the age classes and in two cases (S. araneus, Kington Grove, copper and S. minutus, Wetmoor zinc) the immatures have significantly higher concentrations. Tables 6.16, 6.17, 6.18, 6.19 show similar information for the livers and kidneys.

Another point of note is that the differences in body weight between immatures and matures are not always significant, which is surprising. Goyer et al. (1970) recorded decreased body weight as one of the first effects of lead on the physiology of rats. However analysis of variance for body weights for S. araneus showed no significant differences

Table 6.14

RESULTS OF t-TESTS BETWEEN METAL CONCENTRATIONS ($\mu g g^{-1}$ DRY WEIGHT) IN THE 'REST OF BODY' COMPONENT OF IMMATURE AND MATURE OF S. ARANEUS

SITE	IMMATURES					MATURES				
	Wt	Cd	Cu	Pb	Zn	Wt	Cd	Cu	Pb	Zn
WM	1.701 ±0.087	2.052 ±0.872	11.86 ±0.972	4.671 ±0.714	117.46 ±3.28	2.516ns ±0.386	4.58* ±1.01	13.12ns ±1.36	6.8ns ±2.19	133.07ns ±5.99
n	8	8	8	8	8	3	3	3	3	3
KW	1.635 ±0.042	1.628 ±0.266	12.253 ±0.21	6.477 ±0.422	127.57 ±2.1	2.43 ** ±0.204	5.272*** ±0.817	12.51ns ±1.94	7.62ns ±1.95	141.3* ±4.57
n	39	39	39	39	39	6	6	6	6	6
KG	1.557 ±0.051	2.606 ±0.439	13.67 ±0.57	10.48 ±0.97	128.76 ±3.9	2.53ns ±0.311	3.58ns ±1.19	11.183* ±0.526	2.751ns ±0.801	131.57ns ±5.02
n	33	33	33	33	33	3	3	3	3	3
TP	1.583 ±0.048	4.51 ±0.99	13.638 ±0.433	17.26 ±1.58	147.62 ±4.91	2.529*** ±0.207	8.65*** ±1.09	14.762ns ±0.754	20.74ns ±4.37	154.12ns ±2.92
n	29	29	29	29	29	9	9	9	9	9
PW	1.601 ±0.0496	4.441 ±0.808	14.027 ±0.731	20.64 ±0.96	140.6 ±5.99	2.805** ±0.239	233.3*** ±36.9	15.37ns ±1.35	34.86*** ±2.79	163.8ns ±18.3
n	24	24	24	24	24	5	5	5	5	5
HW	1.352 ±0.119	77.6 ±67.2	21.22 ±1.93	66.6 ±14.5	185.0 ±20.5	2.436** ±0.281	326.3** ±47.0	21.71ns ±2.44	89.8ns ±19.9	183.5ns ±15.0
n	7	7	7	7	7	4	4	4	4	4

Results shown as superscripts in the mature columns. Immature mean ± s.e. given for comparison.

ns = not significant. * = p 0.05. ** = p 0.01. *** = p 0.001.

Table 6.15

RESULTS OF t-TESTS BETWEEN METAL CONCENTRATIONS (μgg^{-1} DRY WEIGHT) IN THE 'REST OF BODY' IN IMMATURE AND MATURE S. MINUTUS

SITE	IMMATURES					MATURES				
	Wt	Cd	Cu	Pb	Zn	Wt	Cd	Cu	Pb	Zn
WM	0.7075 ±0.019	2.309 ±0.283	14.120 ±0.648	7.856 ±0.703	131.81 ±6.21	1.282** ±0.117	3.774ns ±0.717	14.12ns ±1.48	8.305ns ±0.615	104.12** ±4.55
n	13	13	13	13	13	6	6	6	6	6
KW	0.7653 ±0.029	1.015 ±0.305	12.313 ±0.45	8.414 ±0.655	115.31 ±3.46	1.171*** ±0.109	2.065*** ±0.243	13.113ns ±0.613	8.738ns ±0.534	118.77ns ±2.36
n	18	18	18	18	18	15	15	15	15	15
KG	0.712 ±0.0124	1.717 ±0.269	15.323 ±0.681	11.059 ±0.608	125.11 ±2.71	0.956** ±0.0719	3.229*** ±0.415	15.336ns ±0.822	13.502** ±0.958	124.55ns ±4.72
n	43	43	43	43	43	18	18	18	18	18
TP	0.895 ±0.963	2.896 ±0.801	13.79 ±0.516	15.66 ±1.67	128.77 ±3.62	1.148** ±0.630	5.521*** ±0.488	15.999*** ±0.417	21.17ns ±3.14	123.25ns ±5.38
n	18	18	18	18	18	18	7	7	7	7
PW	0.745 ±0.0153	4.315 ±0.588	15.363 ±0.43	28.48 ±1.46	134.89 ±3.32	1.184*** ±0.336	7.998*** ±0.45	16.512ns ±0.565	31.86ns ±1.56	136.68ns ±4.52
n	27	27	27	27	27	25	25	25	25	25
HW	0.709 ±0.094	4.29 ±1.13	20.1 ±4.32	91.7 ±10.3	150.5 ±13.9	0.93ns ±0.173	12.00** ±3.59	20.911** ±0.371	102.42* ±4.07	191.1ns ±22.2
n	6	6	6	6	6	4	4	4	4	4

Results shown as superscript in the mature columns. Immature mean ± s.e. given for comparison.

ns = not significant. * = p 0.05. ** = p 0.01. *** = p 0.001.

Table 6.16

RESULTS OF t-TESTS BETWEEN METAL CONCENTRATIONS ($\mu g g^{-1}$ DRY WEIGHT) IN THE LIVERS OF IMMATURE AND MATURE S. ARANEUS

SITE	IMMATURE				MATURE			
	Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn
WM	7.37 ±5.27	14.82 ±2.86	1.736 ±0.96	84.1 ±15.0	14.36* ±0.76	22.9ns ±5.03	0.48ns ±0.25	96.74ns ±8.63
n	7	7	7	7	3	3	3	3
KW	4.3 ±0.817	11.58 ±0.929	1.513 ±0.452	75.25 ±4.96	46.2*** ±12.9	23.24*** ±2.79	2.05* ±0.64	126.8*** ±14.0
n	37	37	37	37	6	6	6	6
KG	11.87 ±5.22	23.25 ±7.4	8.65 ±5.89	249 ±142	14.9ns ±12.6	11.19ns ±3.95	0.49ns ±0.34	54.3ns ±20.7
n	19	19	19	19	3	3	3	3
TP	13.49 ±2.63	14.67 ±1.26	3.927 ±0.655	139.9 ±34.6	68.0** ±19.2	20.85** ±1.71	5.57ns ±1.09	142.5ns ±14.4
n	30	30	30	30	10	10	10	10
PW	9.11 ±2.04	9.57 ±1.10	2.572 ±0.567	73.3 ±6.99	164.0*** ±53.0	28.75*** ±5.3	5.14ns ±1.28	145.3*** ±16.6
n	19	19	19	19	4	5	5	5
HW	111.8 ±45.0	32.5 ±5.65	15.32 ±4.61	158.3 ±17.5	452ns ±407	23.32ns ±1.39	41.8ns ±30.6	196.5ns ±59.6
n	9	9	9	9	2	2	2	2

Table 6.16 contd

SITE	IMMATURE				MATURE			
	Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn
F	12.5	4.73	2.97	1.33	6.63	2.13	6.45	3.68
df	5/115	5/115	5/15	5/15	5/22	5/23	5/23	5/23
p	0.01**	0.01**	0.05*	0.05ns	0.05*	0.05*	0.01**	0.05*

Results shown as superscripts in the mature columns. Immature mean $\pm$ s.e. given for comparison. ns = not significant. * = p 0.05. ** = p 0.01. *** = p 0.001.

Table 6.17

RESULTS OF t-TESTS BETWEEN METAL CONCENTRATIONS ($\mu g g^{-1}$ DRY WEIGHT) IN THE LIVERS OF IMMATURE AND MATURE S. MINUTUS

SITE	IMMATURE Cd	Cu	Pb	Zn	MATURE Cd	Cu	Pb	Zn
WM	5.147 ±0.863	12.45 ±1.66	1.3 ±0.201	110.0 ±11.8	13.15ns ±2.82	15.04ns ±2.86	0.87ns ±0.197	87.35ns ±5.9
n	11	11	11	11	6	6	6	6
KW	2.224 ±0.429	11.61 ±1.73	2.658 ±0.827	62.54 ±7.91	7.37** ±1.5	14.5ns ±2.89	1.846ns ±0.347	96.03* ±8.4
n	18	18	18	18	7	7	7	7
KG	3.25 ±0.584	25.52 ±6.09	2.239 ±0.819	136.4 ±51.8	10.72** ±3.01a	18.74ns ±3.61	2.6ns ±0.504	87.76ns ±8.38
n	30	30	30	30	14	14	14	14
TP	7.36 ±1.76	15.21 ±1.91	2.75 ±0.598	100.4 ±11.1	22.7** ±2.65	21.7** ±1.41	3.114ns ±0.563	115.5ns ±12.3
n	14	14	14	14	7	7	7	7
PW	12.58 ±2.45	7.51 ±1.1	5.44 ±1.79	85.2 ±16.3	29.57** ±3.41	14.89*** ±1.7	4.186ns ±0.645	99.67ns ±7.42
n	18	18	18	18	27	27	27	27
HW	25.03 ±8.47	31.8 ±13.0	6.26 ±2.01	94.27 ±9.31	63.7ns ±23.3	19.46ns ±3.35	13.98ns ±5.23	120.7ns ±12.4
n	5	5	5	5	4	4	4	4

Results shown as superscripts in the mature columns. Immature mean± s.e. given for comparison. ns = not significant. * = p 0.05. ** = p 0.01. *** = p 0.001.

Table 6.18

RESULTS OF t-TESTS BETWEEN METAL CONCENTRATIONS ($\mu g g^{-1}$ DRY WEIGHT) IN THE KIDNEYS OF IMMATURE AND MATURE S. ARANEUS

SITE	IMMATURES					MATURES				
	Wt	Cd	Cu	Pb	Zn	Wt	Cd	Cu	Pb	Zn
WM	0.0183 ±0.0016	6.67 ±4.44	6.52 ±2.07	2.217 ±0.345	58.56 ±8.91	0.069* ±0.0021	10.88* ±1.17	8.802ns ±0.984	1.804ns ±0.952	76.96ns ±3.31
n	6	7	7	7	7	3	3	3	3	3
KW	0.0198 ±0.002	3.616 ±0.689	6.732 ±0.991	2.95 ±0.353	54.01 ±5.83	0.0177ns ±0.0021	37.0*** ±15.3	9.79ns ±2.41	9.67*** ±2.33	137.4** ±34.3
n	36	36	36	36	36	6	6	6	6	6
KG	0.0348 ±0.0215	7.66 ±3.08	10.92 ±1.89	5.239 ±0.827	108.4 ±10.2	0.028ns ±0.007	9.28ns ±4.92	10.83ns ±4.84	1.912ns ±0.675	55.1ns ±25.7
n	19	19	19	19	19	3	3	3	3	3
TP	0.0152 ±0.00196	19.81 ±5.58	6.05 ±1.22	13.26 ±3.6	155.8 ±38.2	0.019ns ±0.0032	41.3ns ±12.7	7.76ns ±1.86	19.26* ±3.79	122.9ns ±15.5
n	30	30	30	30	30	30	10	10	10	10
PW	0.029 ±0.0058	10.28 ±2.8	2.959 ±0.534	5.49 ±1.11	47.2 ±10.8	0.0229ns ±0.0045	154.2*** ±37.0	13.91*** ±2.59	20.08*** ±3.68	109.33*** ±8.17
n	19	19	19	19	19	5	5	5	5	5
HW	0.0474 ±0.0336	86.7 ±37.8	19.27 ±4.44	38.7 ±13.6	143.0 ±27.9	0.0216ns ±0.0048	142.3ns ±98.7	13.65ns ±4.27	380ns ±358	200.9ns ±81.1
n	9	9	9	9	9	2	2	2	2	2

Results shown as superscripts in the mature columns. Immatures mean± s.e. given for comparison.

ns = not significant. * = p 0.05. ** = p 0.01. *** = p 0.001.

Table 6.19

RESULTS OF t-TESTS BETWEEN METAL CONCENTRATIONS (μgg^{-1} DRY WEIGHT) IN THE KIDNEYS OF IMMATURE AND MATURE S. MINUTUS

SITE	IMMATURES					MATURES				
	Wt	Cd	Cu	Pb	Zn	Wt	Cd	Cu	Pb	Zn
WM	0.0099 ±0.00175	5.471 ±0.851	9.12 ±2.07	2.33 ±1.15	135.7 ±27.5	0.0144* +0.0014	7.91ns ±1.61	9.56ns ±5.69	42.0* ±40.5	79.9ns ±20.4
n	11	11	11	11	11	6	6	6	6	6
KW	0.0105 ±0.0013	1.986 ±0.515	5.76 ±1.46	2.577 ±0.501	29.56 ±8.01	0.0132ns ±0.00164	5.92** ±1.47a	4.77ns ±1.43	2.046ns ±0.581	71.5** ±16.6
n	17	17	17	17	17	8	8	8	8	8
KG	0.0092 ±0.0014	2.46 ±0.458	17.8 ±2.98	5.253 ±0.85	97.6 ±12.4	0.0111ns ±0.0023	7.95* ±2.5	16.78ns ±5.79	4.904ns ±0.977	109.0ns ±17.6
n	32	32	32	32	32	11	11	11	11	11
TP	0.0095 ±0.0011	4.15 ±1.07	13.0 ±3.95	3.777 ±0.553	101.6 ±11.7	0.0127* ±0.0014	12.142* ±0.926	3.77ns ±2.69	3.866ns ±0.986	94.87ns ±9.69
n	14	14	14	14	14	6	6	6	6	6
PW	0.0565 ±0.043	9.27 ±1.92	2.87 ±1.15	5.53 ±1.61	66.8 ±16.1	0.0165ns ±0.002	18.76* ±2.46	4.95ns ±1.46	6.75ns ±1.02	73.79ns ±9.49
n	18	18	18	18	18	27	27	27	27	27
HW	0.0138 ±0.00197	15.81 ±5.7	18.2 ±14.2	12.39 ±3.52	78.2 ±12.9	0.0156ns ±0.0046	49.9ns ±19.3	14.03ns ±4.54	51.0ns ±25.9	152.8ns ±52.8
n	5	5	5	5	5	4	4	4	4	4

Results shown as superscripts in the mature columns. Immature mean ± s.e given for comparison.

n.s. not significant. * p 0.05. ** p 0.01. *** p 0.001.

between sites, neither did *S. minutus*, (Tables 6.5 and 6.6) so it is unlikely that the heavy metal pollution is causing a reduction in body weight at polluted sites.

6.3c iv. *Difference in metal concentrations between the two species of Sorex*.

By referring to Tables 6.5 and 6.8 it can be seen that there appear to be some differences in concentrations, particularly of cadmium, between the two species of *Sorex*. Table 6.20 shows the results of t-tests between the species for the two most polluted sites. In general there is no significant difference except for cadmium in mature animals. This suggests that either cadmium is toxic to *S. minutus* at lower levels, or this species is able to control cadmium to some extent, or that some features of its diet expose it to lower levels than *S. araneus*. This last possibility seems most likely.

S. araneus has been shown to accumulate large amounts of cadmium (Andrews *et al*. 1984, Hunter *et al*. 1987c) but comparisons with *S. minutus* have not been made, although cadmium and lead were both detectable in *S. minutus* in unpolluted woodland (Fangmier & Steubing 1986).

Many studies of the diet of both species of *Sorex* have been made. It has been recorded that the diet of *S. minutus*

Table 6.20

t-TESTS FOR DIFFERENCES IN METAL CONCENTRATION BETWEEN S. ARANEUS AND S. MINUTUS

('REST OF BODY' COMPONENTS)

SITE	MATURITY	CADMIUM	COPPER	LEAD	ZINC
PW	Immature	t=0.09 df 47.3	t=1.64 df 30.1	t=3.54 df 36.4	t=-0.57 df 38.3
		p = 0.93 ns	p = 0.11 ns	p = 0.0011 **	p = 0.57 ns
PW	Mature	t=20.92 df 5.1	t=0.74 df 5.4	t=-1.11 df 8.8	t=-1.47 df 4.7
		p = 0.0 ***	p = 0.49 ns	p = 0.30 ns	p = 0.22 ns
HAW	Immature	t=-2.29 df 8.1	t=-0.61 df 7.3	t=1.55 df 9.9	t=-1.13 df 11.0
		p = 0.051 ns	p = 0.56 ns	p = 0.16 ns	p = 0.28 ns
HAW	Mature	t=-9.63 df 4.2	t=-0.16 df 3.1	t=0.088 df 3.2	t=0.21 df 5.3
		p = 0.0006 ***	p = 0.89 ns	p = 0.44 ns	p = 0.84 ns

Calculated on logged data.

corresponds well with pitfall trapped invertebrates, whilst that of S. araneus does not (Pernetta 1976, Churchfield 1982). Dietary competition appears to be avoided, S. minutus feeding on smaller arthropods, less than 5mm in length, consisting of staphalinid beetles, linyphiid spiders and small harvestmen (Pernetta 1976). S. araneus takes food items more than 5mm long; during the summer months these consist of a wide variety of spiders, beetles, harvestmen, snails and isopods (Rudge 1968, Churchfield 1982). In the winter, this species becomes more subterranean and earthworms are taken, these becoming the most important part of the diet (Pernetta 1976). Pernetta (1973) estimated that earthworms may form 30% of the annual diet of S. araneus.

Hunter et al. (1987c) recorded concentration factors for cadmium of greater than one between S. araneus and its diet, which was estimated from faecal pellets (concentrations in the diet were found from pitfall trapped invertebrates) close to a copper refinery. It is interesting that earthworms were not considered part of the diet for the reason that they were 'virtually absent' from the grassland studied. Thus, the diet was found to consist of insect larvae, adult Coleoptera and Arachnida. The high concentrations of cadmium in S. araneus in the study by Hunter et al. (1987c) was considered to be due to the efficient digestion of the food. The whole prey is generally eaten and the assimilation efficiency is increased by coprophagy (Loxton et al. 1975). Oligochaetes were reported to have a seasonal peak in activity in March when caught in pitfall traps by Hunter *et al*. (1987b) at the same site, so it is surprising the *S. araneus* did not eat them.

Worms have been shown to accumulate cadmium (see section 5.1 for more details). The mean cadmium concentration in worms from Haw was found to be 235 $\mu g\ g^{-1}$ and Hunter *et al*. (1987b) record that oligochaetes are second only to isopods in concentration of cadmium. Thus earthworms are potentially a large source of cadmium available to *S. araneus* but not to *S. minutus*.

Both species of shrew have a one year life cycle and the whole population is renewed each year (Crowcroft 1956). The life span is a maximum of 16 months (Shillito 1963) which consists of a summer and winter as an immature and, as maturity is reached in the spring of the following year, one summer as an adult. Thus all mature *S. araneus* have over wintered, spending a large proportion of the time deeper in the ground, feeding predominantly on earthworms. The worms at Haw have particularly high levels of cadmium in their tissues and the shrews will in addition ingest the gut contents of the worms which increases the cadmium intake further. *S. minutus* over winters at the same stage in the life history, but this species remains more surface active and continues to feed on a variety of beetles and other insects (Grainger & Fairley 1978). It is interesting that a large component of the diet of *S. araneus* in the study by

Hunter et al. (1987c) was lycosid spiders which are absent from Haw.

Thus it appears that the greater concentrations of cadmium in mature S. araneus in comparison to S. minutus may be explained in terms of the over wintering diet. S. araneus in winter feeding on earthworms which contain higher concentrations than the arthropods which S. minutus feeds on.

6.3d. Differences in metal concentrations between several species of small mammals.

Tockington Park, although not a heavily contaminated site yielded 6 of the 7 mammal species recorded (A. flavicolis was absent). Although some were present in only small numbers it is of interest to compare metal concentrations between the species. The data are presented in Table 6.21 together with the results of the analyses of variance. Differences are shown between the species, which, when followed by t-tests, show that the two Sorex species are different from the other mammals. Both S. araneus and S. minutus have higher concentrations of cadmium and lead than all the other species examined.

It has been noted before that shrews accumulate cadmium (Hunter & Johnson 1982, Andrews et al. 1984) and lead (Getz et al. 1977) to a greater extent than other small mammals

Table 6.21

METAL CONCENTRATIONS ($\mu g g^{-1}$ DRY WEIGHT) IN THE 'REST OF BODY' COMPONENT OF MAMMALS FROM TOCKINGTON PARK

SPECIES	n	Cd	Cu	Pb	Zn
Clethrionomys glareolus	4	0.38 ± 0.28^{a}	9.09 ± 0.61^{b}	3.84 ± 1.11^{a}	96.26 ± 3.61^{ab}
Apodemus sylvaticus	2	0.99 ± 0.06^{a}	11.99 ± 0.97^{a}	3.74 ± 2.24^{a}	106.76 ± 4.76^{a}
Microtus agrestis	6	0.69 ± 0.13^{a}	16.47 ± 0.91^{ab}	5.4 ± 0.96^{abc}	101.86 ± 7.96^{acd}
Neomys fodiens +	1	0.43	20.68	8.84	112.02
Sorex araneus	9	8.65 ± 1.09^{b}	14.76 ± 0.75^{b}	20.74 ± 4.37^{b}	154.12 ± 2.92^{cb}
Sorex minutus	7	5.52 ± 0.49^{c}	15.99 ± 0.42^{b}	21.17 ± 3.14^{c}	123.25 ± 5.39^{d}
Analysis of variance F		35.96	15.94	11.5	19.75
df		4/23	4/23	4/23	4/23
p		<0.01**	<0.01**	<0.01**	<0.01**

+ Not included in statistical comparisons. Superscripts show results of subsequent t-tests. Any two means with the same letter are not significantly different from each other.

and this is also true for North American species Blarina brevicauda (Quarles 1974, Goldsmith & Scanlon 1977). The reason for this is probably due to the diet, shrews being carnivorous, in comparison to mice and voles which are herbivorous or omnivorous. In the present study a third species of shrew, the water shrew, Neomys fodiens was represented, albeit in low numbers. The limited data available suggest that cadmium and lead concentrations are closer to those of other small mammal species than other shrews. N. fodiens has a similar life cycle to the Sorex species (Price 1953) and feeds on insects, amphibians and small fish (Corbet & Southern 1977). It is also nomadic and is usually found near water. The absence of water from the wood at Tockington Park might suggest that the individual caught was not a resident. If it usually feeds in water it may be subjected to different pollution pressures from the Sorex species.

Microtus agrestis feeds principally on grasses (Evans 1973) although mosses, herbs and grass seeds are also consumed (Ferns 1976). High lead concentrations, even above S. araneus have been recorded in this species found on mine spoil heaps (Roberts & Johnson 1978, Roberts et al. 1978) however other studies in Britain (Andrews et al. 1984, Beardsley et al. 1978) and those including similar American species, Microtus pennsylvanicus (Anthony & Kozlowski 1982, Smith & Rongstad 1982) indicate that Microtus species do not generally accumulate metals to the same extent as shrews. This is shown in the present study.

Low metal accumulation is indicated by the data for _C. glareolus_ which has even lower concentrations than _M. agrestis_ at Tockington Park. _C. glareolus_ is also principally a herbivore but consumes seeds, fruit, fungi, roots and dead leaves as well as fresh plant material (Watts 1968). Very low metal concentrations have been recorded in this species from other polluted sites (Jefferies & French 1972, Williamson & Evans 1972).

6.4 General discussion.

In view of the small number of species and individuals of small mammal found at Haw it is surprising that the two species present were the insectivorous _S. araneus_ and _S. minutus_ which are those containing the largest concentrations of lead and cadmium. Diet is obviously an important factor influencing the uptake and accumulation of various metals by different species, but physiology and metabolism are also influential (Roberts & Johnson 1978). Behaviour and home range size is also important particularly in lead polluted areas beside roads (Welch & Dick 1975, Quarles 1974).

The relatively high levels of pollution at Haw may be limiting or restricting the population. It was considered that lead might limit numbers of the American shrew *Blarina brevicauda* along a roadside verge (Quarles 1974). In the same study life span was recorded to be shortened in a mouse species *Peromyscus maniculatus*. Metals may also have effects on the reproductive capacity, for example injected cadmium causes testicular necrosis in rats (Parizek & Zahor 1956). In the study by Andrews *et al*. (1984) all the *S. araneus* captured at a mine site were immature and it was suggested that damage to the tissues may have affected the reproductive performance of the animals. Corbet (1975) notes that all common small mammal species have an enormous capacity to replace any population loss and it is therefore likely that the populations in polluted areas will be continually supplemented by surrounding populations even if the breeding capacity of 'residents' is severely reduced. However, in a large polluted area such as Avonmouth, if small animals are present they are most likely to have been born there.

Thus, whilst it is difficult to prove from field data, there are indications that the populations of small mammals are reduced at Haw wood. Those that are present appear to have increased concentrations of metals, particularly cadmium. It is possible that high levels of heavy metal pollution causes problems related to reproduction and survival of the animals.

6.5 SUMMARY.

1. 7 species of small mammal were caught in pitfall traps intended for invertebrates.

2. The lowest number of species (2) and individuals (11) were caught at the most polluted site. Neither the number of species nor the number of individuals were significantly correlated with degree of pollution.

3. In half of the sites *S. minutus* was caught in larger numbers than *S. araneus*. This has not been recorded before in woodland, *S. araneus* usually occurs in much greater proportions.

4. Metal concentrations in species other than *Sorex* did not vary significantly between the sites, but none were captured from the most polluted wood.

5. Metal concentrations in *S. araneus* and *S. minutus* did vary significantly between the sites. Cadmium and lead were particularly high in animals from Haw.

6. In general the cadmium concentration was highest in the livers and kidneys of *Sorex* species, copper was highest in the liver and lead and zinc were greatest in the ´rest of body´ compartment which included the bones.

7. The cadmium concentrations increased greatest with age in S. araneus. Differences were also found in S. minutus. Other metals did not show consistent increases.

8. Mature S. araneus have significantly higher cadmium concentrations than than mature S. minutus. Other metals were not consistently different. This may be due to the different diets over the winter before attaining maturity. S. araneus feeds predominantly on earthworms during the winter whilst S. minutus continues to feed on small arthropods. Earthworms contain high levels of cadmium in their tissues and gut contents, both of which are consumed by S. araneus.

9. The two Sorex species contain higher levels of cadmium and lead than the other small mammals even at a less polluted site. This is probably due to their mainly carnivorous diet. The water shrew, Neomys fodiens appears to be closer in metal levels to the herbivorous and omnivorous mice and voles than the carnivorous Sorex species.

Chapter 7.

THE EFFECT OF HEAVY METAL POLLUTION ON THE INVERTEBRATE COMMUNITIES.

7.1 Introduction.

The long term pitfall trapping was intended as a method for catching and examining the macroarthropod community. Whilst collections commenced on 2.1.85 and ended on 23.3.86 due to unforseen circumstances (principally trap vandalism and weather conditions), a rather shorter period of time existed where all trapping occasions were comparable for all sites. This period (25.4.85 to 2.1.86) was taken as the standard trapping period and was used in the statistical analyses. The results discussed are those for the animals identified to species level. These consisted principally of spiders, carabid beetles, millipedes, centipedes, harvestmen, woodlice and to a lesser extent pitfall trapped molluscs, worms and mammals.

7.2 Using log-normal plots to identify pollution indicator species.

7.2a Introduction.

It has been shown for many different populations that if a large sample is taken and the cumulative percentage of species recorded is plotted against the number of individuals per species (grouped into geometric classes) a bell shaped normal distribution results. This distribution is called log normal. If the cumulative percentage is plotted on probability paper a straight line is produced. Such plots have been used to illustrate changes in populations due to pollution (Gray & Mirza 1979, Gray 1979). In a marine environment, Gray & Mirza (1979) showed that unpolluted sites showed the typical straight line. When under slight pollution, (the transition stage) a larger number of geometric classes was covered and the plot showed a distinct break in the line. Under severe pollution a straight line was resumed, but the slope was shallower than the pre-pollution stage and the number of classes increased. This is illustrated in Figure 7.1. The break in the plot in the transition stage was considered to be due to an increase in numbers of ´middle-order´ organisms when the population suffered slight pollution. Larger numbers of organisms have been observed at low levels of pollution in terrestrial situations too (for example Alstad et al. 1982). The increased numbers of geometric classes covered with increasing pollution is probably due to the increased dominance of a few species (Gray 1979). The species represented at the break in the line at the transition stage have been suggested as good indicator species (Gray 1979). Further discussion of the methods is given in Gray (1981). Obviously, any type of disturbance to the community might generate such patterns, not just pollution.

7.2 Methods and results.

Data from the pitfall captures were sorted so that plots could be made. Spiders were first plotted on their own, then all the main groups of arthropods identified to species level were lumped together. An example of the data plotted is shown in Table 7.1.

The graphs for the spiders alone are shown in Figure 7.2. For the four sites showing low levels of heavy metal pollution an approximately straight line is produced. For site 5 (Pegwell wood),the plot shows a distinct break at group VI. Site 6 (Haw wood) is rather straighter. Both these sites show an increase in the number of classes covered from the other four. When all the arthropods are lumped together (Figure 7.3) a similar break in the line is produced for site 5 (Pegwell wood) again at class VI. The number of geometric classes does not increase at the more polluted sites for these data.

Several of the plots show the first point (class I) to be out of line. This is considered to be unimportant and is due to the difficulty of correctly identifying all the rare individuals (Gray & Mirza 1979).

An attempt was made to test for significant differences between the curves. This was done using a Kolmogorov-

Details of figures on the following four pages.

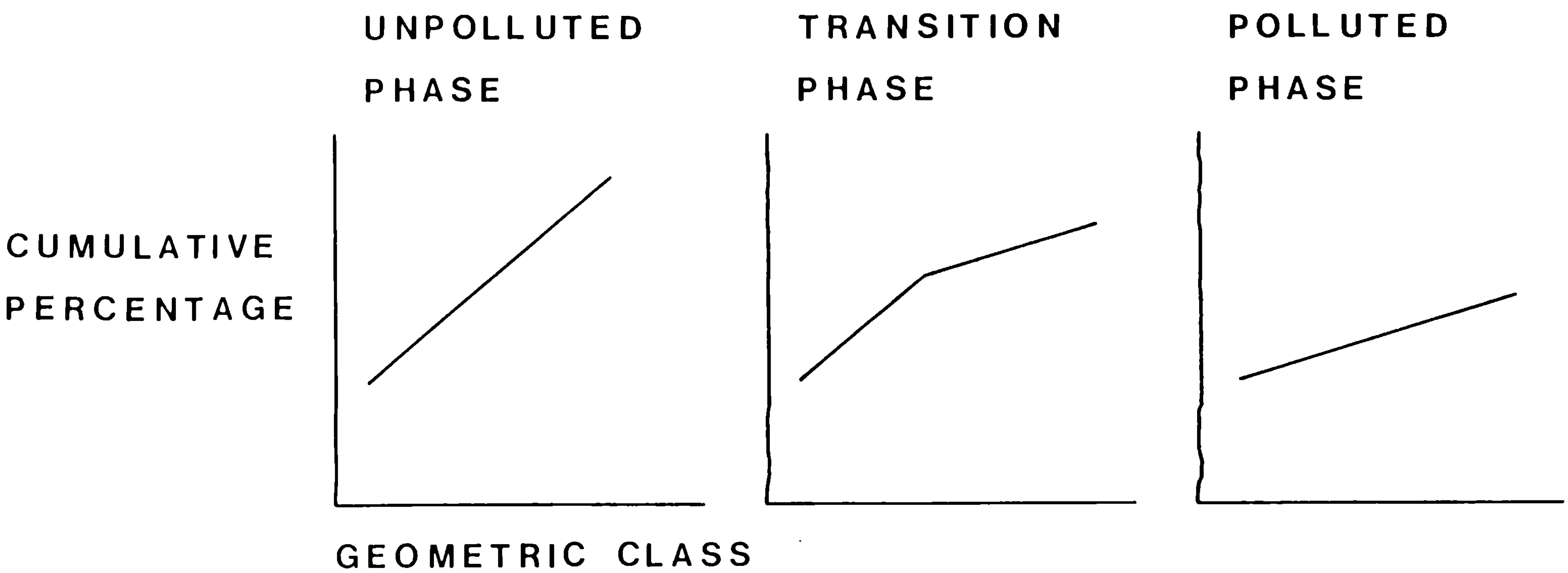
Figure 7.1 Ideal log normal plots to illustrate the effect of pollution.

Figure 7.2 Graphs to show log-normal plots for spider data for each site. Drawn for spider data. Cumulative percentage on a probability scale is plotted against the geometric class.

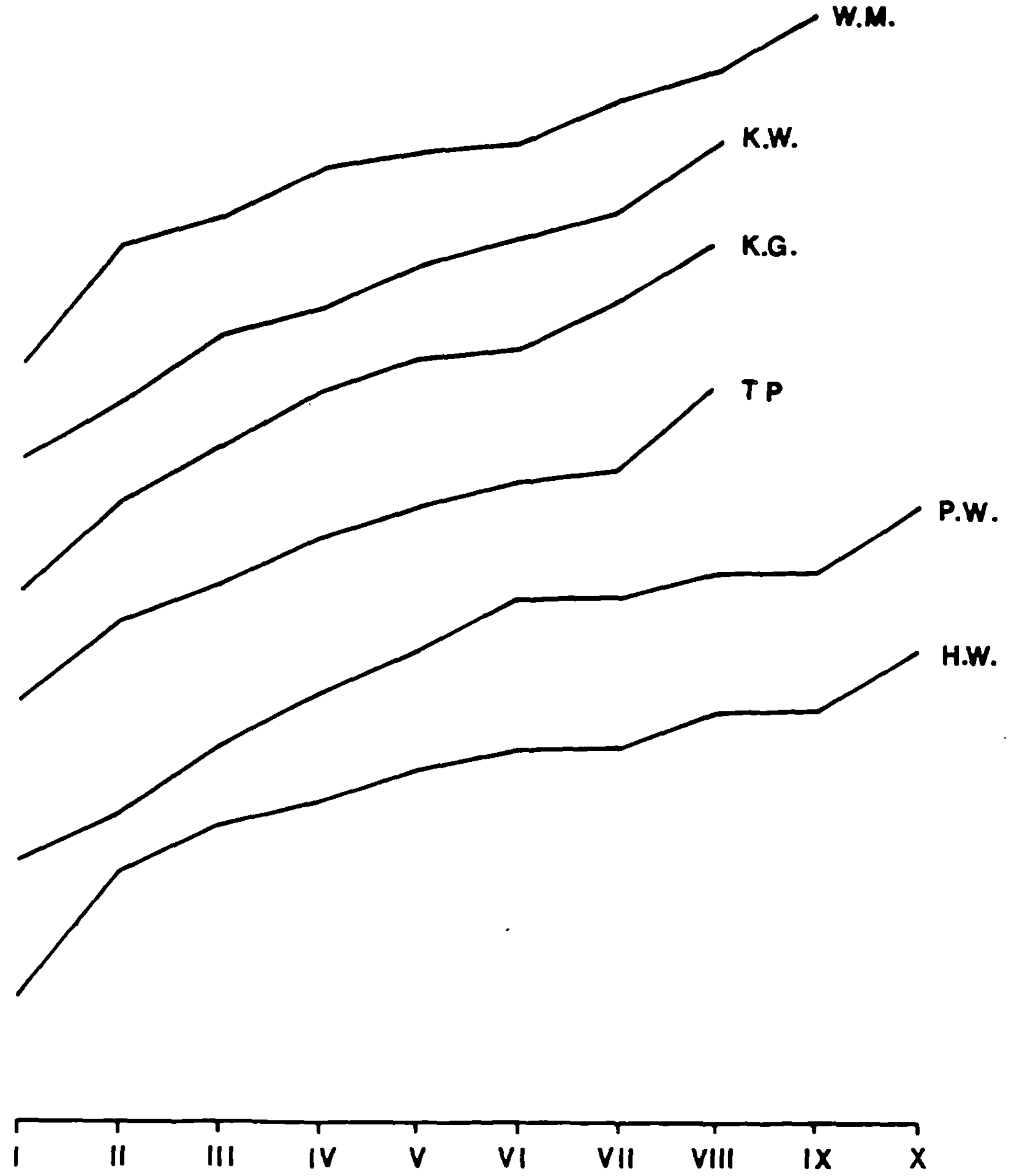
Figure 7.3 Graph to show log-normal plots for total arthropods for each site.

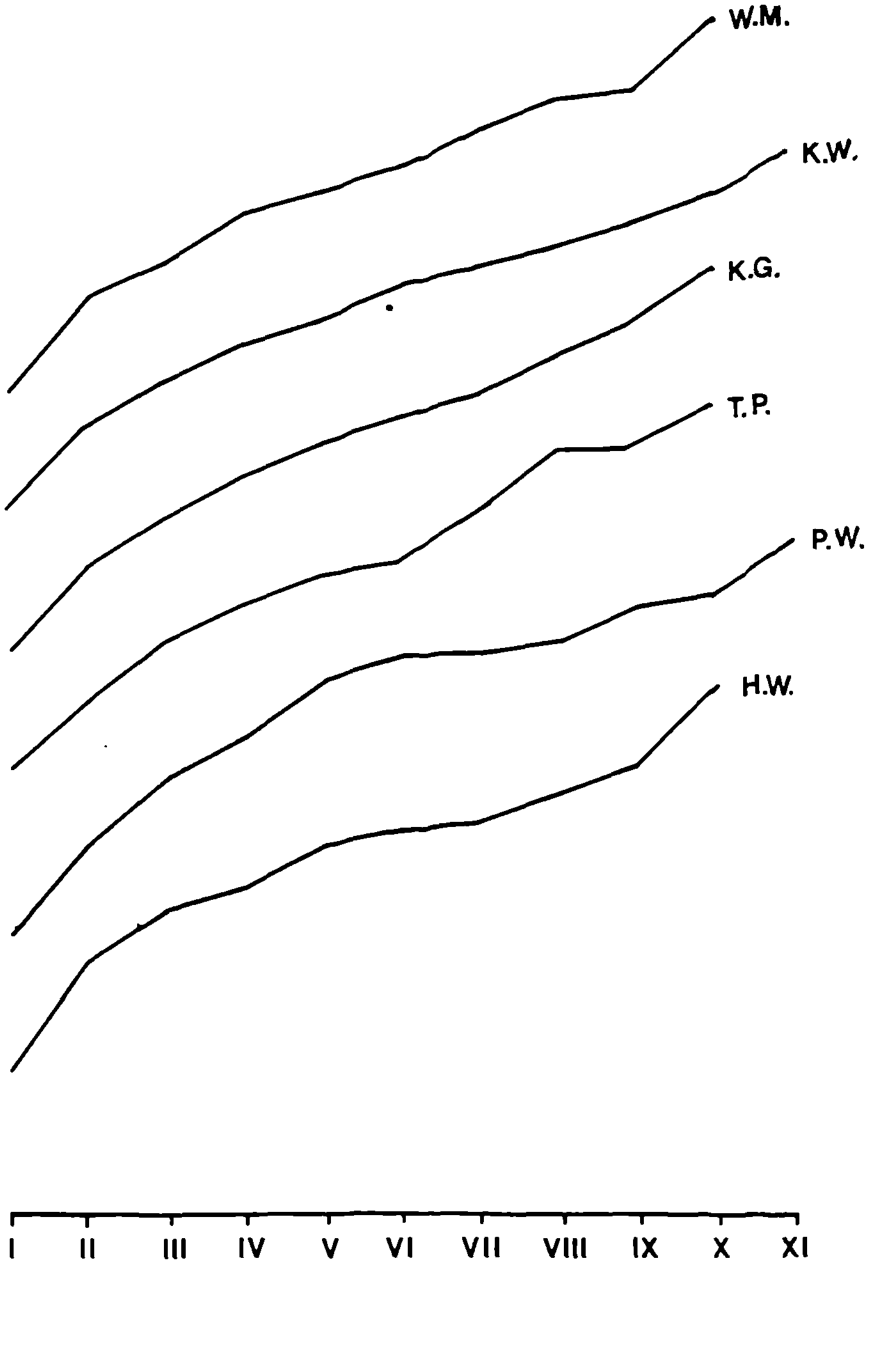
Figure 7.4 Graph to show log-normal plots for data from Bengtsson & Rundgren (1984). Sites are marked as in the original paper.

THEORETICAL LOG-NORMAL PLOTS.



From Gray & Mirza 1979


W.M.
K.W.
K.G.
T P
P.W.
H.W.
I
II
III
IV
V
VI
VII
VIII
IX
X


W.M.
K.W.
K.G.
T.P.
P.W.
H.W.
I
II
III
IV
V
VI
VII
VIII
IX
X
XI

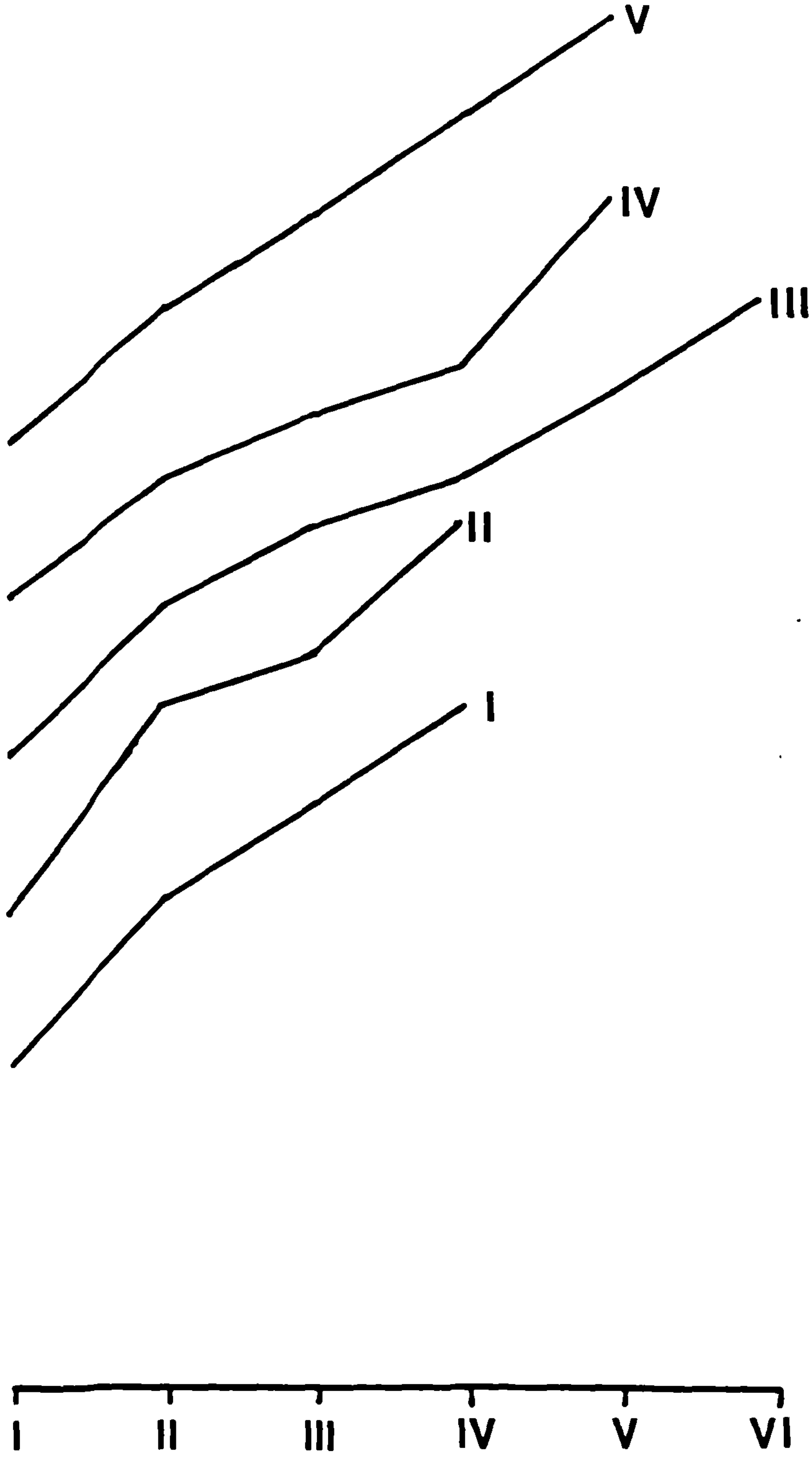

V
IV
III
II
I
I
II
III
IV
V
VI

Table 7.1

DATA USED FOR PLOTTING A LOG-NORMAL DISTRIBUTION.

SPIDERS CAUGHT FROM SITE 1 (WETMOOR)

Geometric Class	Arithmetic class i.e. no of indiv. species	No of Species	No of Species as % of total	Cumulative %
I	1	15	23.8	23.8
II	2-3	18	28.6	52.4
III	4-7	8	12.7	65.1
IV	8-15	10	15.9	81.0
V	16-31	3	4.8	85.8
VI	32-63	1	1.6	87.4
VII	64-127	5	7.9	95.3
VIII	128-255	2	3.1	98.4
IX	256-511	1	1.6	100

Smirnov two sample test. This showed that none of the differences were significant at $p<0.05$. The plots of spider data for sites 5 and 6 (Pegwell wood and Haw wood) were however significantly different at $p<0.1$.

7.2c Discussion and conclusions.

The plots drawn show a resemblance to those given by Gray & Mirza (1979). According to the graphs, site 5 (Pegwell wood) is at the transition stage, receiving levels of pollution which are affecting the relative abundance of the arthropods.

Gray & Mirza (1979) suggested the use of the Kolmogorov-Smirnov test, however they did not compute it for their sites. Due to the absence of raw data for more than one site and the fact that the plots were drawn on probability paper it is difficult to calculate Kolmogorov-Smirnov test using their data. It is probable that many of the original plots are not significantly different. Thus obtaining results similar to those obtained using the present data.

Although the Kolmogorov-Smirnov tests did not show large significant differences the plots may still prove of use. The class at the break point of the line (class VI in this instance) may contain useful indicator species. Those in this class are shown in Table 7.2.

Table 7.2

POSSIBLE INDICATOR SPECIES DETERMINED FROM THE LOG - NORMAL PLOTS. (PEGWELL WOOD, CLASS VI)

Linyphiid Spiders	Diplocephalus picinus
	Lepthyphantes flavipes
	Lepthyphantes pallidus
	Microneta viaria
Harvestmen	Anelasmocephalus cambridgii
	Rilaena triangularis
Millipede	Julus scandinavius
Carabid beetles	Pterostichus madidus
	Leistus ferrugineus

The spiders are all linyphiids, and therefore difficult to determine without a microscope. The millipede species, Julus scandinavius when immature or female is difficult to distinguish from Ophyiulus pilosus. Of the beetles, Pterostichus madidus is fairly easy to identify by eye and is widespread, but the two harvestmen may be the most useful. Anelasmocephalus cambridgii is unmistakable (except from young Trogulus tricarinatus which is rare and only found in a few southern counties). Rilaena triangularis can be determined with a hand lens, and in addition is widespread in Britain. Some of these species may prove to be useful indicators of heavy metal pollution.

The method illustrated here has, in the past been used for marine communities. There is no reason why it should not be equally applicable to terrestrial environments although it does not appear to have been tried. It is interesting that Bengtsson & Rundgren (1984) plotted similar graphs for beetles caught by pitfall trapping at the Swedish sites near Gusum. By testing for differences between the curves by Kolmogorov-Smirnov tests they recorded significant differences in the beetle communities from some sites. However, they did not draw the graphs on probability paper and made no mention of the work by Gray & Mirza. Replotting of the graphs of Bengtsson & Rundgren (1984) onto probability paper produces the results in Figure 7.4 (page 243). The number of geometric classes covered is considerably less than the plots produced by Gray & Mirza (1979) or by the present study. The lines for sites I, II and V are almost straight, those for sites III and IV show a step, particularly site IV. Site I is closest to the brass mill and V is furthest away. That showing the clearest step, site IV, is a similar distance from the source of pollution as Haw wood and similar in levels of copper, although falling between Kington Grove and Knapp Wood in terms of lead and zinc concentrations. It is possible that this is showing a transition stage; however similar shaped curves were produced from several sites in the present study when only the first three months trapping data was used. It is possible that the numbers of animals caught in the Swedish study are not large enough to show the effect of the pollution in this format. This also illustrates that the Kolmogorov-Smirnov test may indicate differences between the plots drawn for the sites but not necessarily the precise differences indicated by Gray & Mirza (1979).

7.3 Numbers of individuals and species.

The total numbers of macroarthropod species and individuals at each site are illustrated graphically in Figure 7.5. Figure 7.6 shows the numbers of species and individuals of decomposers (including molluscs). The numbers of arthropod species appears to drop with increasing proximity to the smelter. This is not shown in the decomposer species. The number of individuals is far more variable, both total number of species and for decomposers show a similar pattern. A peak in numbers occurs at KW. and a drop at TP. (and WM. for the decomposers). The two most polluted sites (PW. and HW.) show very similar numbers to KG.

These totals and those for the taxonomic groups ie. Diplopods etc. were correlated using the Pearsons product moment correlation coefficient both with each other and with various environmental variables measured at the sites. As many of the environmental data were in the form of metal concentrations which were non normally distributed, they were $\log_{10}$ transformed before correlation. Those results which were significant ie. $r>0.811$, $df.=4$, $p<0.05$ are indicated in Table 7.3.

It must be remembered that approximately 1 in 20 such correlations may prove significant by chance. For part a. of the table alone over 90 correlations were calculated thus 4 may be chance relationships.

Details of figures on the next two pages.

Figure 7.5 Plots of:

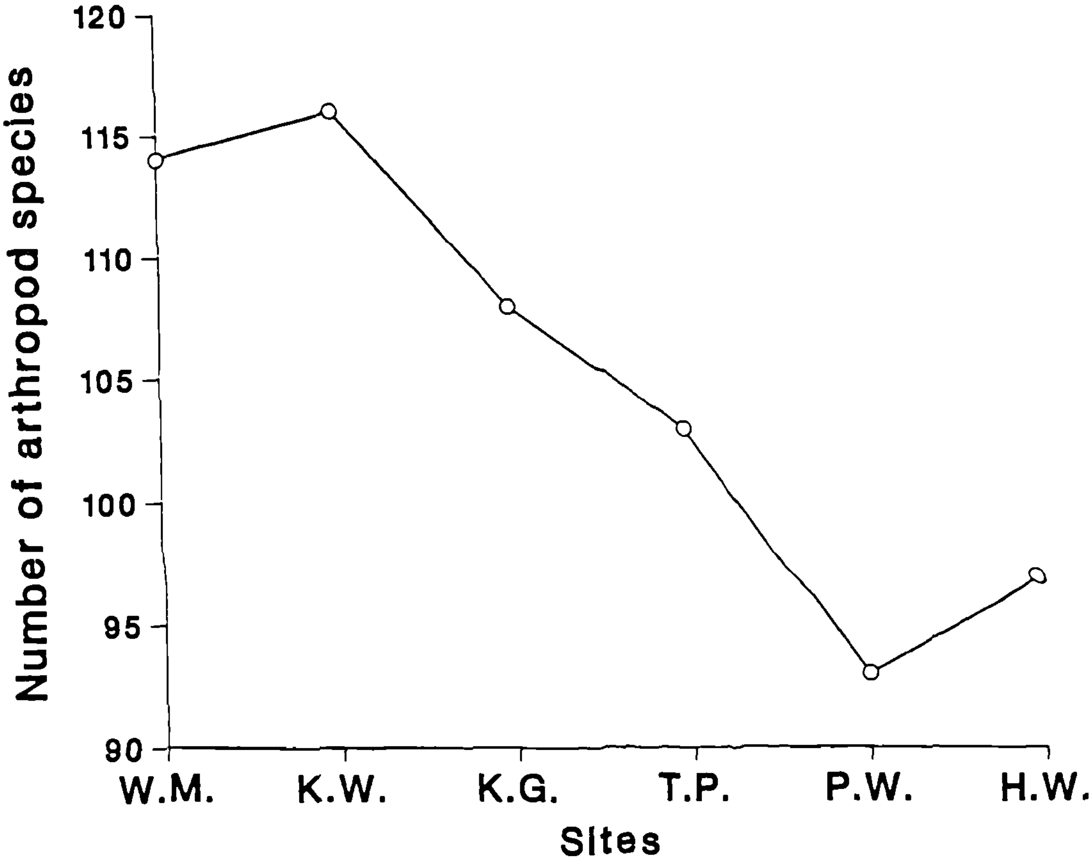
a. Arthropod species at each site

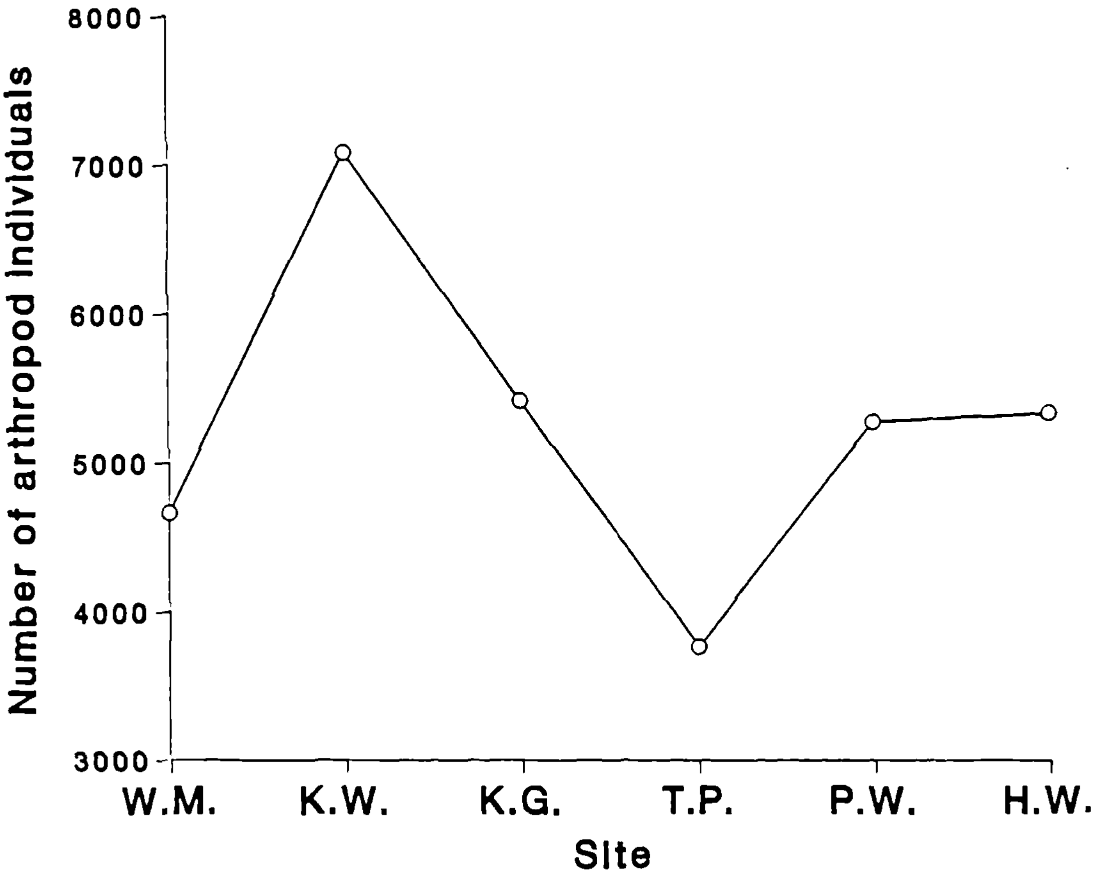
b. Arthropod individuals at each site

Figure 7.6 Plots of:

a. Decomposer species at each site

b. Decomposer individuals at each site


120
115
110
105
100
95
90
Number of arthropod species
W.M.
K.W.
K.G.
T.P.
P.W.
H.W.
Sites


8000
7000
6000
5000
4000
3000
Number of arthropod individuals
W.M.
K.W.
K.G.
T.P.
P.W.
H.W.
Site

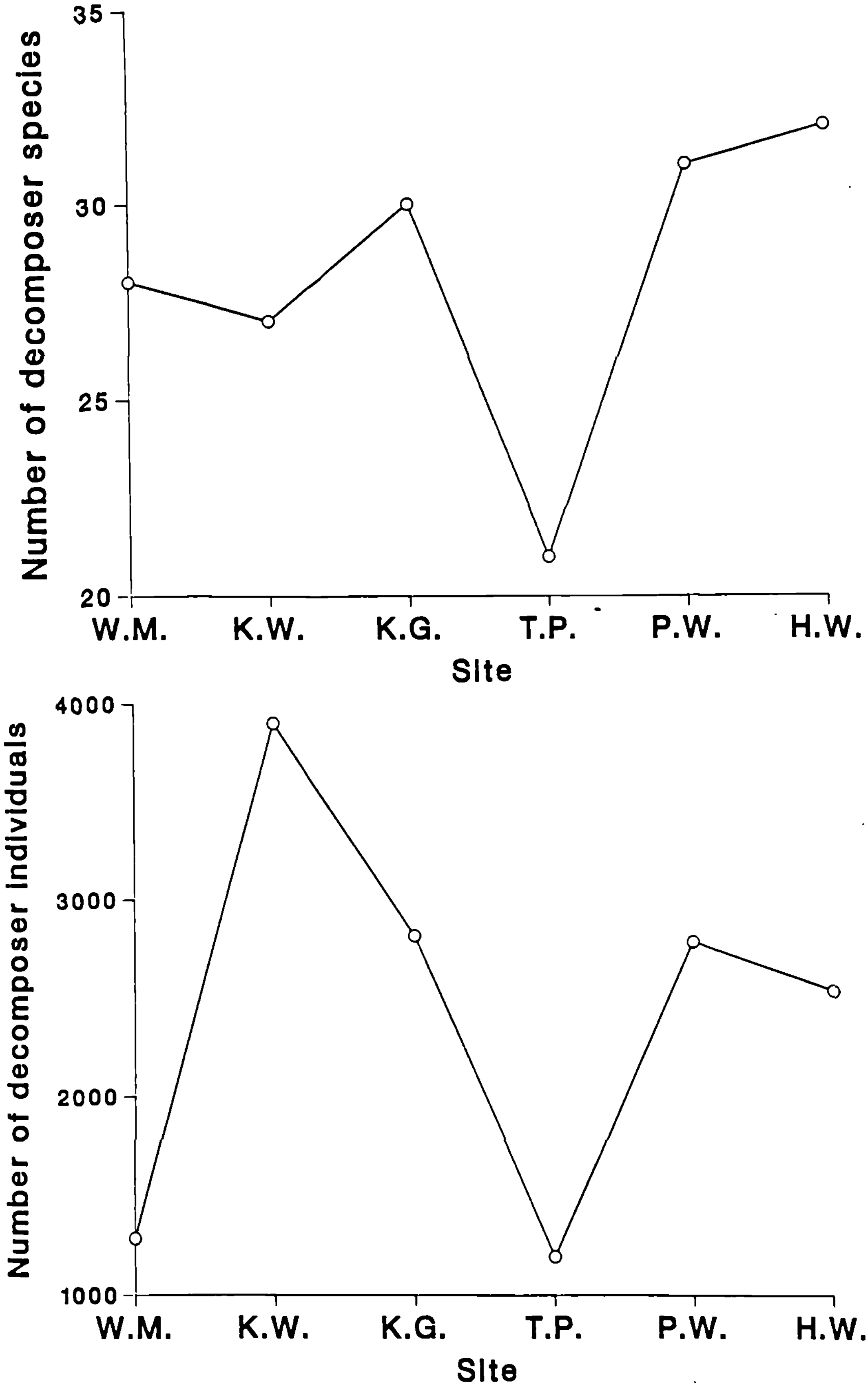

35
30
25
20
Number of decomposer species
W.M.
K.W.
K.G.
T.P.
P.W.
H.W.
Site
4000
3000
2000
1000
Number of decomposer individuals
W.M.
K.W.
K.G.
T.P.
P.W.
H.W.
Site

Table 7.3

SIGNIFICANT CORRELATIONS OBTAINED WHEN CORRELATING:

A) BETWEEN NUMBERS OF SPECIES AND NUMBERS OF INDIVIDUALS IN EACH TAXONOMIC GROUP

Correlation between		r	p
Spider species	Carabid individuals	-0.9223	0.01 **
Spider species	Harvestmen individuals	0.9729	0.01 **
Spider species	Total species	0.8546	0.05 *
Carabid individuals	Centipede individuals	0.9386	0.01 **
Carabid individuals	Harvestmen individuals	-0.8912	0.05 *
Harvestmen species	Mollusc species	0.8273	0.05 *
Harvestmen species	Total species	0.8986	0.05 *
Harvestmen individuals	Decomposer individuals	0.8594	0.05 *

Table 7.3 contd

B) BETWEEN NUMBERS OF SPECIES AND NUMBERS OF INDIVIDUALS IN EACH TAXONOMIC GROUP AND ENVIRONMENTAL VARIABLES

Correlation between		r	p
Spider individuals	pH litter	0.8863	0.05 *
Spider individuals	pH F/H layer	0.8977	0.05 *
Spider individuals	Distance from sea	0.8663	0.05 *
Spider individuals	Density of trees	0.8795	0.05 *
Carabid species	Number of plants	0.8287	0.05 *
Harvestmen species	Cadmium in litter	-0.8954	0.05 *
Harvestmen species	Copper in litter	-0.8733	0.05 *
Harvestmen species	Copper in F/H layer	-0.8619	0.05 *
Harvestmen species	Lead in litter	-0.936	0.01 **
Harvestmen species	Lead in F/H layer	-0.9642	0.01 **
Harvestmen species	Lead in soil	-0.9270	0.01 **
Harvestmen species	Zinc in litter	-0.9003	0.05 *
Harvestmen species	Zinc in F/H Layer	-0.8490	0.05 *
Harvestmen species	Zinc in soil	-0.8529	0.05 *
Harvestment species	Distance from smelter	0.8707	0.05 *
Mollusc species	Lead F/H layer	-0.8273	0.05 *
Total individuals	Altitude	-0.8287	0.05 *

A noticeable feature of part b. of the table is that all the significant correlations with metal concentrations are negative. However, nearly all are correlated with the number of harvestmen species. Again, not too much emphasis should be placed on these results due to the large number of correlation coefficients calculated. Again the large number of significant correlations (at $p<0.05$) should be treated with caution or a more stringent level of significance used (e.g. $p<0.01$).

7.4 Calculation of diversity indices using the data.

7.4a Introduction.

Measurement of the diversity of a community or a taxocene may help in the understanding of its structure. Various diversity indices have been used in ecological studies of invertebrates, for example Anderson (1975), Emberson (1985) and Morris & Rispin (1987), and in pollution studies (Smith *et al*. 1979, Santas 1986). A popular suggestion is that the diversity of a stable community is high (Connell & Orias 1964) and that the diversity is reduced if stress of some kind is applied to the community (Smith *et al* 1979b, Dennis & Patil 1979). Pollution is of course a form of stress, which will have a similar effect (Patil & Taillie 1976). Patil & Taillie (1976) concluded that, "The most suitable means of analysing community structure for the purposes of pollution assessment appears to be the diversity index."

This however is disputed by Colwell (1979) who considers that greater diversity is not related to a more stable environment. No evidence for decreased diversity of plankton was found with increasing copper concentration (Smith et al. 1979b) but Santas (1986) recorded a significant positive correlation between the diversity of Berlese extracted animals and distance from a centre of urbanisation (taken to be a gradient of lead pollution).

An additional problem is the choice of diversity index used. Whilst s, simply the number of species is one possible measure of diversity, it is also useful to weight this by the number of individuals in each species. Two communities may contain the same number of species but the individuals might be distributed very differently between them.

A plethora of diversity indices have been proposed, thus the difficulty is in deciding the most appropriate for the situation. Pielou (1975) states three desirable properties for an index and lists only two indices which possess these properties, the Brillouin index and the Shannon index. The former measures the diversity of a complete small collection whereas the latter measures the diversity of a sample from a large community. Therefore Shannon's (H′) would seem the most appropriate in this situation. DeBenedictis (1973) also favours the use of both s (i.e. number of species) for species richness and H´ as a combined measure of species number and evenness. Peet (1974) considers H´ as the best of this particular type of index. An additional advantage of H´ is that Hutcheson (1970) has given a method for calculating the estimated variance and the indices can then be compared with each other using t-tests. Accordingly both s and H´ have been calculated for the data sets obtained by pitfall trapping. H´ *was computed as follows:-*

$$H' = - p_i \log p_i$$

where pi is the proportion of the community belonging to the ith species.

It has been suggested that H´ should be given together with a measure of evenness, for example J´ (Engen 1979). Measurement of evenness is also recommended by Pielou (1975). The evenness indicates how the individuals are proportioned between the species. J´ has been calculated for the pitfall samples too.

The importance of calculating indices using species data has been shown by Resh (1979) who demonstrated that the indices (H´) calculated using the generic level were significantly lower than those calculated using the specific level.

7.4b Results and discussion.

Table 7.4 shows the various forms of diversity calculated for each group of animals sampled. The following are recorded:- the number of species (s), the number of individuals (n), index of evenness (J´), index of diversity (H´) and the variance of H´ used to calculate the t-tests between diversities.

It is noticeable that some groups, for example the woodlice, were caught in large numbers but represented by only a few species. In contrast, the spiders were caught in similar, or lower, numbers as the woodlice but consisted of many species. This is shown in the diversity indices. H´ for the centipedes, millipedes, woodlice and harvestmen are all in the range 0.4-1.9 ie. low in diversity. The carabids and molluscs have intermediate values of 1.3-2.4. The spiders are by far the most diverse group with values ranging from 2.0-2.7.

The values of J´ are on the whole wide ranging ie. varying from low to high evenness within a taxonomic group. An exception is the spiders which are more constant in the ´middle´ range. A noticeable point is the value of 0.9 (very even) for the molluscs at site KW.

The majority of t-tests for differences between the diversities were significant; these are summarised in Table

Table 7.4

NUMBER OF SPECIES (S) INDIVIDUALS (N), DIVERSITY INDEX (H'), AND EVENNESS (J') FOR VARIOUS GROUPS OF ANIMALS.

A. Millipedes

Site	S	n	J'	H'	Variance of H'
WM	9	154	0.653	1.436	4.888 E-3
KW	8	1014	0.747	1.552	5.843 E-4
KG	12	1020	0.768	1.908	7.058 E-4
TP	8	371	0.791	1.646	7.643 E-4
PW	6	374	0.363	0.65	3.047 E-3
HW	6	1122	0.753	1.349	4.645 E-4

B. Woodlice

Site	S	n	J'	H'	Variance of H'
WM	4	1077	0.784	1.086	3.599 E-4
KW	5	2859	0.558	0.898	1.384 E-4
KG	4	1683	0.709	0.982	3.204 E-4
TP	4	778	0.59	0.818	8.481 E-4
PW	6	2164	0.293	0.526	2.436 E-4
HW	5	1234	0.322	0.518	4.714 E-4

Table 7.4 contd

C. Molluscs

Site	S	n	J'	H'	Variance of H'
WM	15	55	0.597	1.616	4.459 E-2
KW	14	33	0.921	2.43	2.102 E-2
KG	9	120	0.641	1.692	1.526 E-2
TP	9	47	0.624	1.371	3.381 E-2
PW	19	252	0.714	2.101	7.683 E-3
HW	21	185	0.664	2.022	9.398 E-3

D. Harvestmen

Site	S	n	J'	H'	Variance of H'
WM	11	1147	0.627	1.504	9.443 E-4
KW	11	1481	0.531	1.274	5.436 E-4
KG	9	1135	0.609	1.339	5.881 E-4
TP	9	733	0.766	1.684	6.043 E-4
PW	10	392	0.676	1.556	3.023 E-3
HW	7	929	0.363	0.705	1.157 E-3

E. Spiders

Site	S	n	J'	H'	Variance of H'
WM	63	1617	0.648	2.685	1.181 E-3
KW	67	1446	0.646	2.714	1.619 E-3
KG	60	994	0.641	2.623	2.611 E-3
TP	56	1283	0.523	2.106	2.195 E-3
PW	43	1246	0.555	2.088	2.369 E-3
HW	56	1242	0.509	2.049	2.359 E-3

Table 7.4 contd

F. Carabids

Site	S	n	J'	H'	Variance of H'
WM	21	573	0.672	2.046	2.167 E-3
KW	22	250	0.781	2.378	3.569 E-3
KG	18	513	0.653	1.887	3.689 E-3
TP	21	488	0.736	2.24	1.918 E-3
PW	23	985	1.455	1.428	1.799 E-3
HW	18	634	0.648	1.873	1.838 E-3

G. Centipedes

Site	S	n	J'	H'	Variance of H'
WM	6	95	0.507	0.909	1.181 E-2
KW	3	46	0.379	0.417	1.704 E-2
KG	5	83	0.272	0.438	1.366 E-2
TP	5	75	0.587	0.944	1.358 E-2
PW	5	129	0.282	0.454	8.729 E-3
HW	5	116	0.434	0.698	9.292 E-3

7.5. This table also orders the sites on the basis of the diversity indices within each taxocene. It can be seen that for several groups there is a tendency for the two more polluted sites, HW. and PW. to show the lowest diversities.

The diversity indices (H') were correlated with each other and with various environmental variables and the significant results are shown in Table 7.6. The diversity of woodlice is very sensitive to all metals in the soils except lead. The diversity of harvestmen is negatively correlated with cadmium and copper and the spiders with zinc.

The calculation of diversity indices have shown some interesting results. Some groups have shown direct negative relationships between diversity and metal concentrations in the soil. By rank ordering the sites on the basis of the indices, the two most polluted sites do stand clear from the others (Table 7.7).

Connell & Orias (1964) considered greater diversity to occur where there was greater environmental stability and where the rate of energy flow through the food web is fast. The present work appears to agree with this. However, Taillie (1979) has commented that no single index adequately summarises community structure. Thus an additional method was searched for, in order to analyse this species data in more detail. Flores et al. (1979) considered that multivariate techniques should be used when looking at the effect of pollution on communities. Multivariate analysis was then attempted using the available data.

Table 7.5

COMPARISON OF DIVERSITY INDICES FOR ALL GROUPS

Group	HIGHEST H^1 ------------------------> LOWEST H^1					
Millipedes	KG^{c}	TP^{d}	KW^{a}	WM^{ab}	HW^{b}	PW^{e}
Woodlice	WM^{a}	KG^{b}	KW^{a}	TP^{c}	PW^{d}	HW^{e}
Molluscs	KW^{a}	PW^{ab}	HW^{bc}	KG^{d}	WM^{cd}	TP^{d}
Harvestmen	TP^{b}	PW^{c}	WM^{d}	KG^{a}	KW^{a}	HW^{e}
Centipedes	TP^{a}	WM^{a}	HW^{ab}	PW^{b}	KG^{b}	KW^{b}
Carabids	KW^{a}	TP^{a}	WM^{c}	KG^{b}	HW^{b}	PW^{d}
Spiders	KW^{a}	WM^{a}	KG^{a}	TP^{b}	PW^{b}	HW^{b}

Any two sites (along a row) with the same superscript have diversity indices (H^1) which are not significantly different when calculated using t-tests.

Table 7.6

PEARSON PRODUCT MOMENT CORRELATION COEFFICIENTS BETWEEN DIVERSITY INDICES AND VARIOUS ENVIRONMENTAL VARIABLES

Diversity	Environmental variables	r	p
Spider	Zn in F/H Layer	-0.8333	*
Spider	Zn in Soil	-0.8837	*
Spider	Distance from Smelter	0.818	*
Centipede	Soil type	-0.9091	*
Harvestmen	Cd in F/H Layer	-0.8348	*
Harvestmen	Cd in Soil	-0.8453	*
Harvestmen	Cu in Litter	-0.888	*
Harvestmen	Cu in F/H Layer	-0.8133	*
Woodlice	Cd in Litter	-0.8418	*
Woodlice	Cd in F/H Layer	-0.8623	*
Woodlice	Cd in Soil	-0.8262	*
Woodlice	Cu in F/H Layer	-0.8439	*
Woodlice	Cu in Soil	-0.9564	**
Woodlice	Zn in Litter	-0.8355	*
Woodlice	Zn in F/H Layer	-0.8589	*
Woodlice	Zn in Soil	-0.846	*
Woodlice	Distance from Smelter	0.8921	*

Degrees of Freedom = 4 in all cases

Significance levels $p<0.05 = 0.811$

$p<0.01 = 0.917$

$p<0.001 = 0.974$

Table 7.7

RANK ORDER OF THE SIX SITES ACCORDING TO THE DIVERSITY INDICES (H')

For each group of animals, each site is given a rank between 1 & 6. The value of 1 is given to the site with the highest density and 6 to that with the lowest. The total is the sum of all the ranks for all groups at that site.

GROUP	SITE					
	WM	KW	KG	TP	PW	HW
Millipedes	4	3	1	2	6	5
Woodlice	1	3	2	4	5	6
Molluscs	5	1	4	6	2	3
Harvestmen	3	5	4	1	2	6
Centipedes	2	6	5	1	4	3
Carabids	2	1	4	3	6	5
Spiders	2	1	3	4	5	6
TOTAL	19	20	23	21	30	34

7.5 Multivariate analysis of community structure.

7.5a Introduction to methods of multivariate analysis.

There are various methods of relating species abundance to environmental measures. The simplest is to plot species abundance against one or two variables to give a line plot or an isopleth (Whittaker 1967). When many environmental factors have been recorded, multiple regression can be used. However, there are several reasons why this is not the ideal method of analysis. These include the fact that environmental variables may be highly correlated and the species data may contain many zeros, both of which cannot be accommodated by multiple regression. In addition a separate analysis must be carried out for each species or group of species, which can be very laborious (Ter Braak 1987b).

There are now several methods of ordination which can be used in ecological research to simplify large sets of environmental of abundance data. Ordination is the process of arranging samples (or species) in relation to one or more gradients (Whittaker 1967). Perhaps the most commonly encountered method is principal component analysis (PCA).

Reciprocal averaging is an alternative method which was reported by Hill (1973) to be better than principal component analysis for species data, which are not in general normally distributed. The same method was developed almost simultaneously in France where it was called correspondance analysis (CA), by which name it is referred to in this thesis. Species ordination by the correspondance analysis method was considered ´markedly more meaningful than PCA species loadings´ by Hill (1973). A comparison of eight similar types of analysis was reported by Kenkel & Orloci (1986). The general conclusion was that nonmetric multidimensional scaling was the best method, however this is difficult to compute and is not readily available as a computer program. The best of the metric methods and second best overall was correspondance analysis. An outline of how correspondance analysis works is given below, the example is taken from Hill (1973).

In a situation examining the water relations of species and sites, the species can be divided up into those liking wet ´0´ and those liking dry ´100´. Scores for the sites can be found by scoring 0 or 100 for each species present and using the average species score to define the site score. Thus a site with only wet species will have a score of 0 and that with only dry species a score of 100. A site with half and half will score 50. These site scores can then be used to recalibrate the species. The new species scores can be rescaled to fall between 0 and 100 and are used to obtain new site scores and so on. The process is repeated until the scores stabilise and they are then taken as the final ordination. The final score does not depend on the original scores but the number of iterations (repeats) might. Correspondance analysis has been used in a variety of ecological situations; for example, Spaull (1979) used the method to study arthropods on Aldabra atoll.

An extension of correspondence analysis has been developed which extends the original application by relating the ordination further to environmental variables; this method is called canonical correspondance analysis or CCA, (Ter Braak 1986). With CCA the relationship of the sites and the species can be related to measured environmental variables. CCA avoids the limitations of PCA ordination in ecology, which requires normally distributed input data; this cannot be possible with species abundance data. CCA is a novel and relatively untried method, but it seemed particularly appropriate for this application.

A computer program, CANOCO (Ter Braak 1987a) has been developed to compute canonical correspondance analysis as well as other related and simpler analyses. This has been used for examining groups of plant species (Ter Braak 1987b) and animals (Ter Braak 1986). One of the options offered by the program is computation of detrended CCA; this is a method of overcoming the arch effect in CA, which is when the second ordination axis is strongly related parabolically to the first. The detrended version also rescales the axes. Wartenberg et al. (1987) have shown that detrending may distort the data; it is certainly not better than the original version and is possibly worse.

The computer program CANOCO was obtained and has been used to analyse the pitfall trapping data. CCA uses the sites and the species to produce weighted averages of each other (as described above) and then relates the resulting ordination to environmental variables. The result is a new set of ordination axes which are produced by taking into consideration the species, sites and environmental factors. Canoco produces considerable output in the form of eigenvalues, species, sites and environmental scores, correlation matrices etc. These can be used to produce biplots using an associated program CANOPLOT, which helps in data interpretation. A separate program written by J. M. V. Rayner was used to process the output of CANOCO to plot environmental variables, species and site scores on the same biplot. The biplots show only two dimensions, but there are as many axes in the ordination as there are environmental variables. Therefore the true picture is multi-dimensional and each species will exist in a particular place in a hypervolume. Each biplot is essentially a two-dimensional projection of this volume. A major advantage of CCA is that the axes are generated in order of their significance, so that it is rarely necessary to consider more than the first two or three axes.

Within a single community it might be expected that each species would occupy its own individual space; if enough samples were taken and sufficient environmental factors measured, this might be revealed by using the CCA method.

7.5b Methods.

The pitfall trapping results were used to run Canoco. Animals caught between collection 8 (25.4.85) and collection 26 (2.1.86) were used because for this period the trapping times were comparable for all sites, i.e. no traps were destroyed. A species data set was constructed which treated each ´group´ as a species. Mostly taxonomic groups were used, for example Isopoda, Chilopoda etc. Two exceptions to this were beetle larvae, which were classed separately from the adults, and ´insects´, which encompassed all those insects not covered by other groups. The remaining species data sets consisted of taxonomic groups, with individuals identified down to species level. Thus a few of the original grouped data are not represented in this form (for example Diptera). The pooled data set is all the individual species (those identified) lumped together.

The environmental data consisted of metal concentrations (Cd, Cu, Pb, Zn) and pH for each of 3 soil layers, details of vegetation and more general features of each of the six woods (area, altitude etc.).

7.5c Ordination of the sites using environmental data (and selection of environmental data for use in CCA).

In section 4.3d various methods were used to classify the woodland sites. Mathematical ordination provides another method. First principal components analysis was carried out on a subset of the environmental data, consisting of the metal concentrations alone. The resulting eigenvalue for the first axis of ordination was 0.995, implying that a very large proportion of the variation (99.5%) between sites can be explained by the first axis of ordination. The biplot (Figure 7.7a) shows that one site (HW, site 6) the most polluted site, stands away from the others which are all clustered together. To resolve differences between these sites the PCA was repeated with the same set of environmental data (the metal concentrations) transformed to natural logarithms. The first ordination axis is still very strong, but the biplot this time (Figure 7.7b) reveals differences between the sites more clearly. Site 6 (Haw wood) is still a long distance from the others along the axis of the first principal component, but site 5 (Pegwell) now also stands apart at an intermediate level of metal concentration. The remaining sites may be ordered 3,2,4,1 on the basis of the first principal component of metal concentrations. Log transforming the metal concentrations helps to produce a clearer picture with better separation of

Figure 7.7 PCA on metal data from each site.

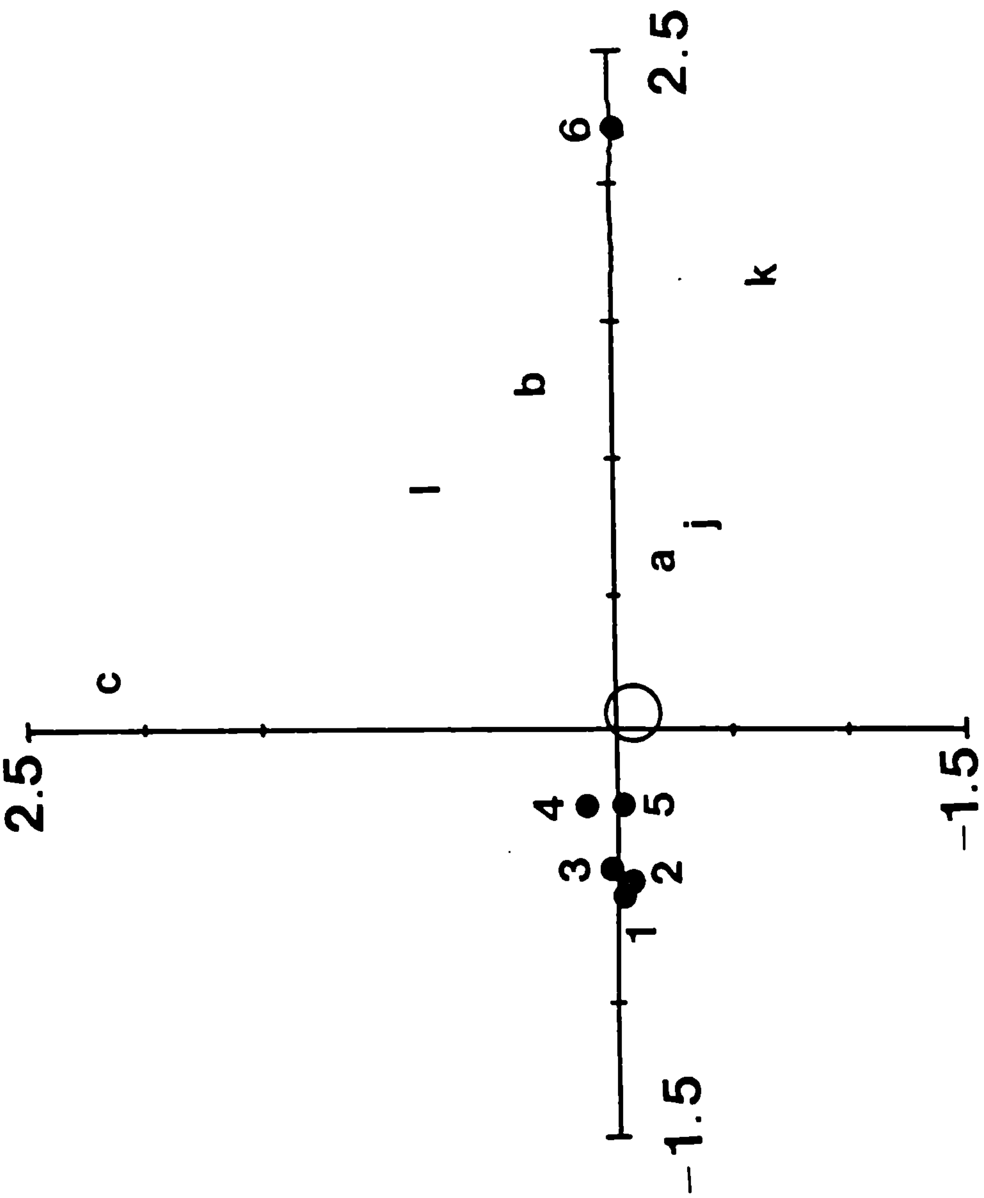
7.7a Axis 1 horizontal, axis 2 vertical, data not transformed.
7.7b Axis 1 horizontal, axis 2 vertical, data log transformed

Legend.
Sites:
1 Wetmoor Wood
2 Knapp Wood
3 Kington Grove
4 Tockington Park
5 Pegwell Wood
6 Haw Wood

Environmental variables:

a Pb litter	g Cd litter
b Pb F/H	h Cd F/H
c Pb soil	i Cd soil
d Cu litter	j Zn litter
e Cu F/H	k Zn F/H
f Cu soil	l Zn soil

In 7.7a the circle represents all variables not indicated elsewhere on the diagram.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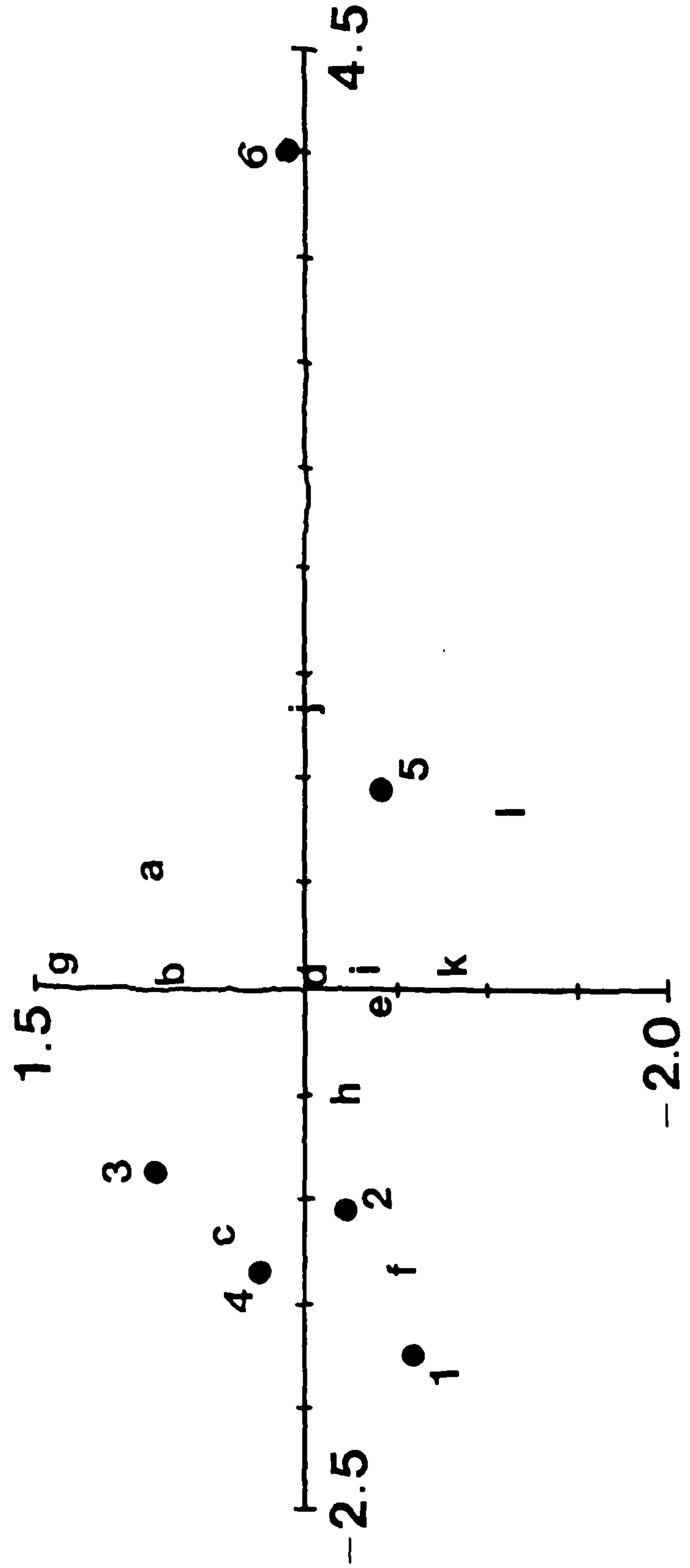
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the sites. This is adopted for the remainder of the analysis.

PCA was also calculated for the remaining environmental variables (log transformed) which are shown in Figure 7.8. These variables are widely scattered and the eigenvalue of the first axis was 0.884.

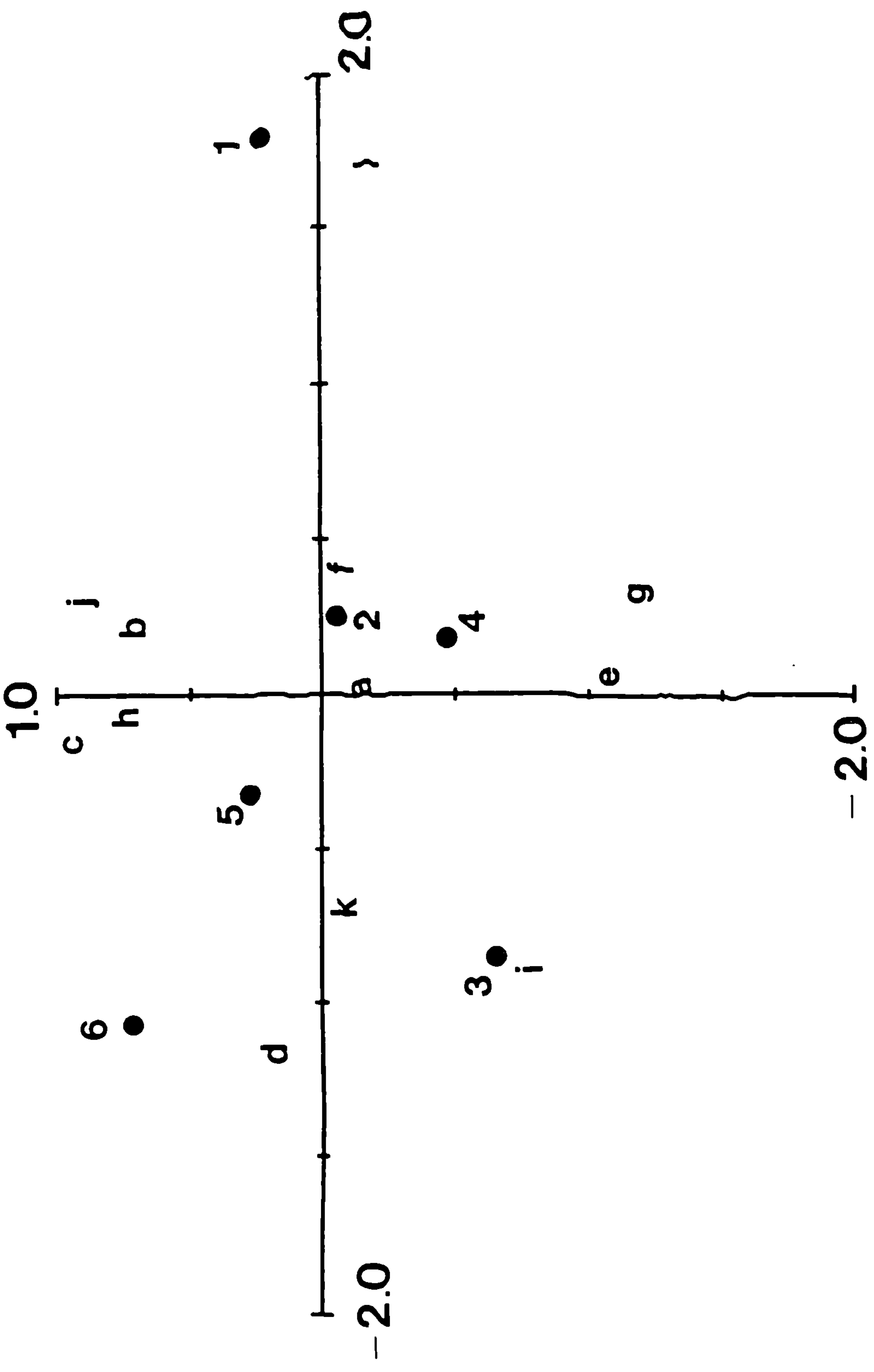
As a test, CCA was computed using the grouped data set as species data and all the environmental variables (Figure 7.9). The ordination diagram clearly shows that the majority of the metals act in a similar way and there is some redundancy in the data. This is not surprising in view of the magnitude of the eigenvalue for the metal PCA (Figures 7.7a and b). However, there is a problem with this diagram. Because of the number of environmental variables (24 in this instance) exceeds the number of sites minus 2 (=6-2=4), canonical correspondance analysis cannot be computed. The program switches to ordinary correspondance analysis, and the environmental variables are superimposed onto the biplot but are not used in the calculation of the ordination axes. For the canonical version of the program to work effectively, the number of environmental variables has to be reduced to 4 or less (as the number of sites could not be increased).

First PCA was computed for all the environmental variables (metals and others). The eigenvalues for the first three

Figure 7.8 PCA non metal variables, data log transformed.
Axis 1 horizontal, axis 2 vertical.

Sites as in Figure 7.7
Environmental variables:

a pH litter	g Altitude
b pH F/H	h Area of wood
c pH soil	i Soil type
d litter depth	j Canopy cover
e Distance from smelter	k Density of trees
f Distance from sea	l No. of plant species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j
k

Figure 7.9 CCA using species groups and all environmental variables.

Axis 1 horizontal, axis 2 vertical
Sites as in Figure 7.7
Environmental variables (shown as arrows):

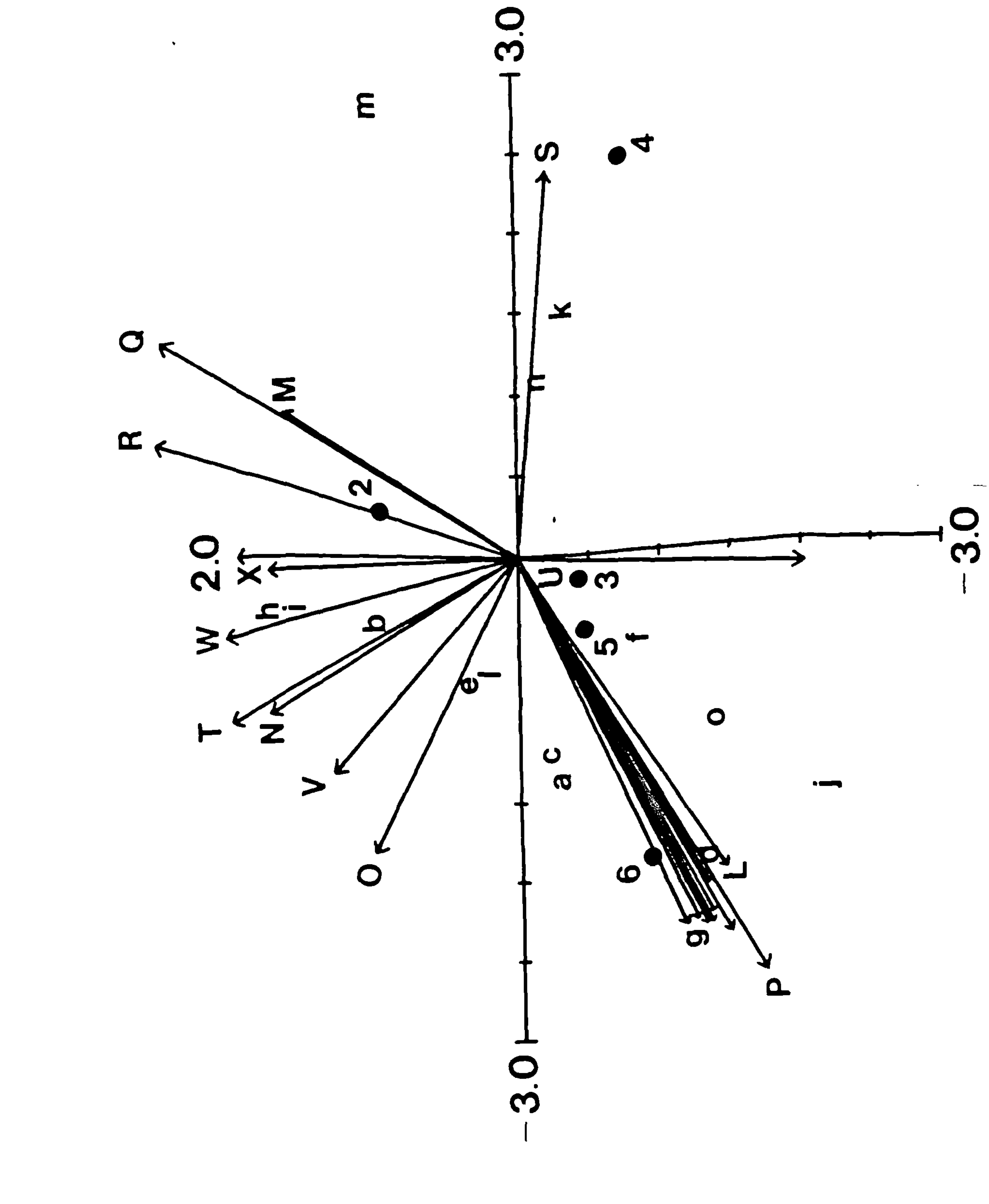
A Cd litter	M pH litter
B Cd F/H	N pH F/H
C Cd soil	O pH soil
D Cu litter	P Litter depth
E Cu F/H	Q Distance from smelter
F Cu soil	R Distance from sea
G Pb litter	S Altitude
H Pb F/H	T Area
I Pb soil	U Soil type
J Zn litter	V Canopy cover
K Zn F/H	W Density of trees
L Zn soil	X No. plant species

All variables not labeled are amongst the large number pointing to the bottom left of the figure.

Species groups:

a Millipedes	i Harvestmen
b Woodlice	j Pseudoscorpions
c Worms	k Diptera
d Molluscs	l Hymenoptera
e Centipedes	m Earwigs
f Beetles	n Mammals
g Beetle larvae	o Insects
h Spiders	

Some species groups are hidden under the bulk of ´arrows´.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axes were 0.76, 0.18 and 0.11 and all three axes are needed to explain the distribution of the sites. The three principal components of the environmental variables are sufficient to explain all significant variation between the sites. The ordination diagrams are shown in Figures 7.10a and b. The 24 variables used in this calculation are given in Table 7.8 with their values for axes 1, 2 and 3. The values indicate the contribution of a particular variable to a particular axis. The larger the number (positive or negative) the greater the influence. The signs indicate the direction of their influence.

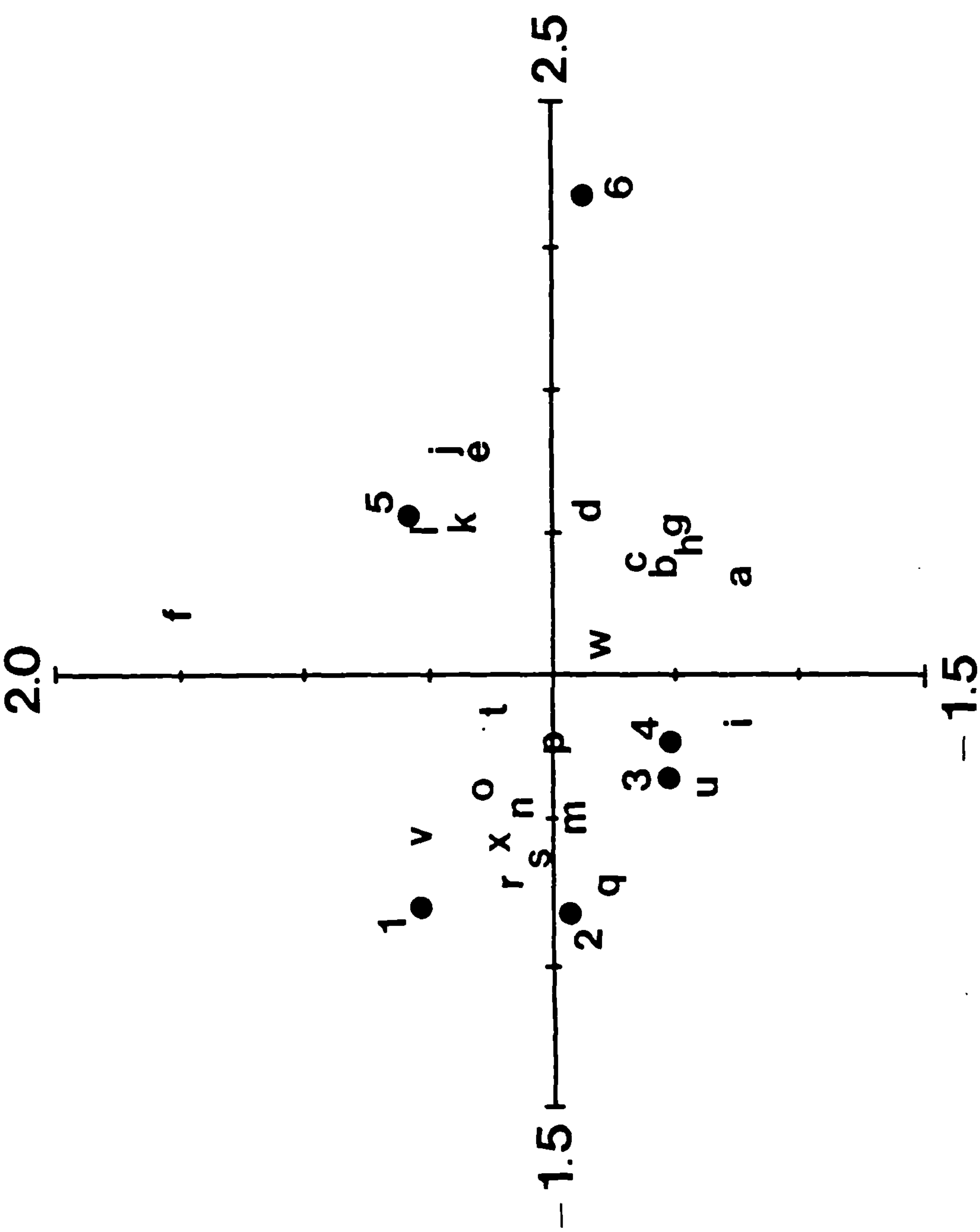
Axis one is mainly governed by metal pollution. Most of the values for metal concentrations are similar and are highest in this axis. Other major contributions to axis 1 are pH, number of plants and canopy cover, which all decrease with increased metal concentrations. Distance from the smelter, distance from the sea and altitude similarly contribute negatively to this axis. Of these it could be expected that distance from the smelter increases with decreasing metal concentrations and, with the exception of lead in the soil layer, there are significant correlations between distance from smelter and all the metals in all the soil layers ($r>-0.951$, $df.=4$, $p<0.01$). Axis two is also principally a metal axis; it is characterised by copper in the soil layer, lead in the soil and cadmium in the litter. The relative amount of copper and lead in the soil may be the important factor. The important contributors to axis three are the soil type

Figure 7.10 PCA of all environmental variables.

7.10a Axis 1 horizontal, axis 2 vertical.
7.10b Axis 1 horizontal, axis 3 vertical.

Sites are as in Figure 7.7
Environmental variables:

a Cd litter	m pH litter
b Cd F/H	n pH F/H
c Cd soil	o pH soil
d Cu litter	p Litter depth
e Cu F/H	q Distance from smelter
f Cu soil	r Distance from sea
g Pb litter	s Altitude
h Pb F/H	t Area
i Pb soil	u Soil type
j Zn litter	v Canopy cover
k Zn F/H	w Density of trees
l Zn soil	x No. plant species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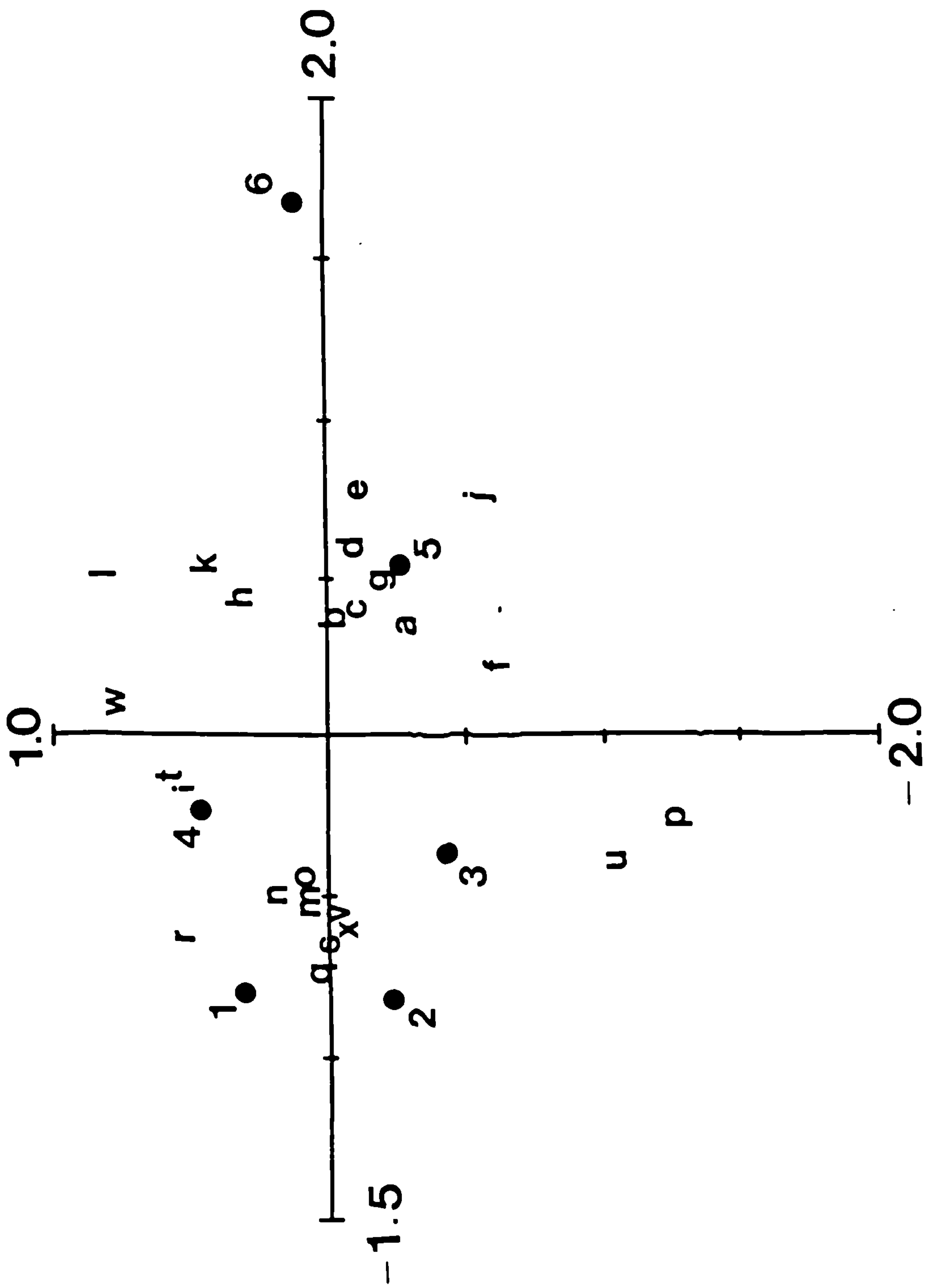
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Table 7.8

RESULTS OF PRINCIPAL COMPONENTS ANALYSIS ON ENVIRONMENTAL VARIABLES (for details see text)

ENVIRONMENTAL VARIABLE	AXIS 1	AXIS 2	AXIS 3
Cd Litter	35	-78	-30
Cd F/H	38	-47	-4
Cd Soil	22	151	-61
Cu Litter	59	-14	-9
Cu F/H	78	35	-12
Cu Soil	22	151	-61
Pb Litter	49	-51	-20
Pb F/H	44	-54	31
Pb Soil	-17	-74	53
Zn Litter	76	39	-56
Zn F/H	54	36	44
Zn Soil	50	52	82
Ph Litter	-51	-8	7
Ph F/H	-49	11	19
Ph Soil	-44	26	9
Area	-14	22	58
Litter Depth	-26	-1	-126
Distance from Smelter	-73	-22	2
Distance from Sea	-63	17	53
Soil Type	-39	-63	-104
Altitude	-64	5	-1
Canopy Cover	-55	54	-2
No. Plant Species	-61	18	-6
Density of Trees	10	-19	77

and litter depth and the density of trees. Zinc in the soil may also be important, but other metal concentration are less so.

Accordingly, the primary principal component axis can be seen as the metal pollution effect, the second axis as a function of lead and copper in the soil, and the third as the effect of trees, soil type and litter depth.

From Figures 7.10a and b (the ordination diagram shown from different angles i.e. axes) considerable information can be deduced about the sites. Sites 3 and 4 are very similar in metal concentrations, but differ in tree density and litter depth (site 4 having high density of trees and low litter depth). Site 6 is considerably higher in metal concentrations (axis 1) than the other sites but is relatively unaffected by the other principal components and is very similar to site 2 in respect of the second axis which is metal related. In conclusion, site 6 (Haw wood) is not obviously different from the other sites in anything other than the first axis which is dominated by the metal concentrations.

To standardise the main portion of the analyses, the first 3 principal component axes from the PCA of all the environmental variables were used as environmental variables for input to CCA. These explain sufficient of the variation in the environmental data and reduction to three quantities permits the full canonical CA to be used.

7.5d CCA on the groups of animals.

Canonical correspondance analysis was computed for the species data grouped taxonomically. The results are shown in Figures 7.11a and b, which show ordination axes 1 and 2 and 1 and 3. (The third viewpoint (axes 2 and 3) is not shown as the information can be gathered from the other two plots). CCA was also performed with the same sets of data to explore the the effects of various options available in the program. For example, the species data can be transformed to log or squareroot and it is possible to down weight rare species. None of the options had any appreciable impact on the results, and unless stated otherwise all further computations were carried out with no transformation of any type. (Environmental variables were already log-transformed in computing the PCA.)

Some points to help in the interpretation of the biplots are given below:-

1. Environmental variables are represented as arrows from the origin, in the line of most effect. Sites are shown as ● and the appropriate number. Species are represented as letters (and sometimes numbers) and/or symbols, keys are

Figure 7.11 CCA using species groups and PCA axes for environmental variables.

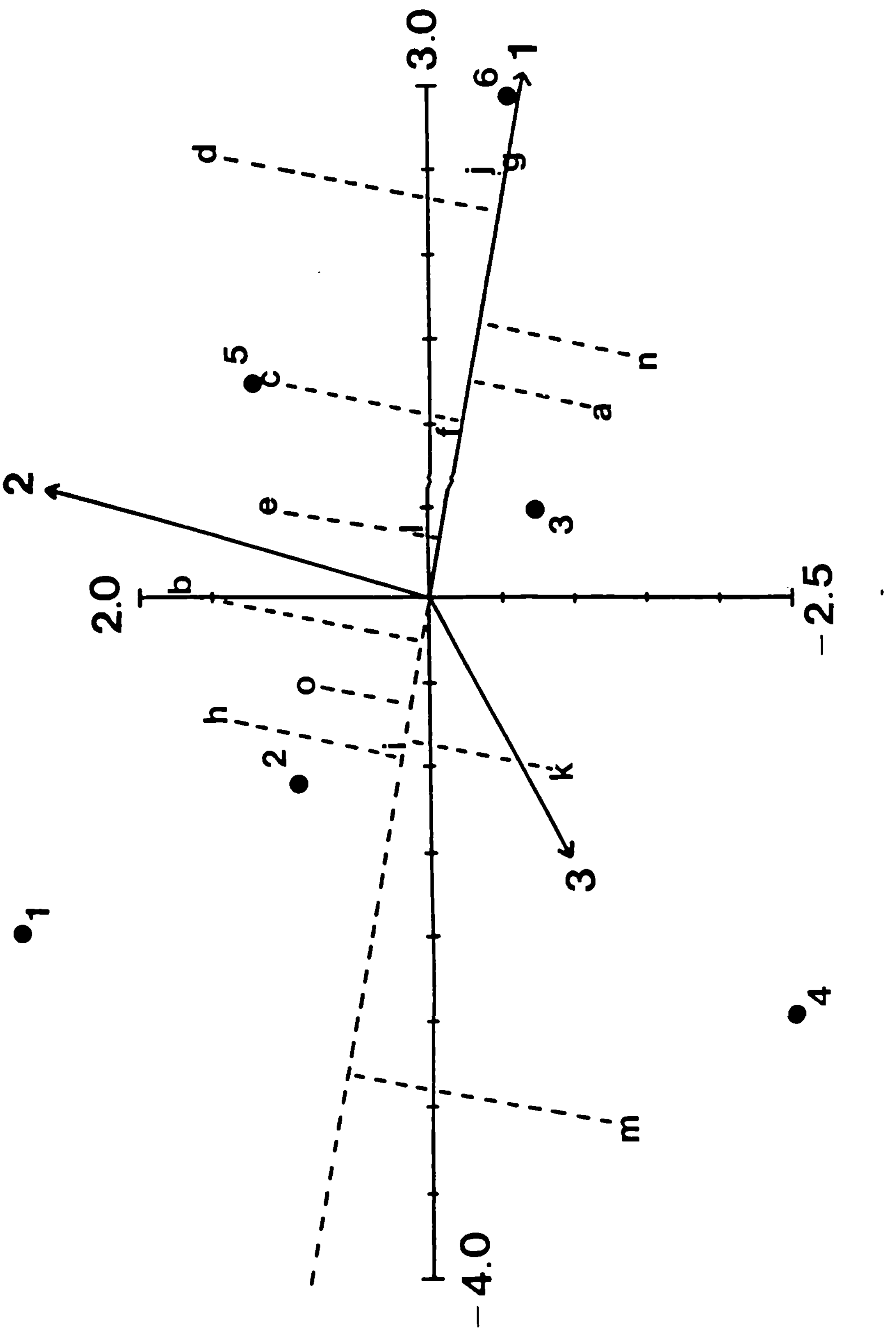
7.11a Axis 1 horizontal, axis 2 vertical. Dotted lines indicate the relationships between the species groups and the first principal component axis.
7.11b Axis 1 horizontal, axis 3 vertical.

sites as in Figure 7.7

Environmental variables:
1 PCA axis 1
2 PCA axis 2
3 PCA axis 3

Species groups:

a	Millipedes	i	Harvestmen
b	Woodlice	j	Pseudoscorpions
c	Worms	k	Diptera
d	Molluscs	l	Hymenoptera
e	Centipedes	m	Earwigs
f	Beetles	n	Mammals
g	Beetle larvae	o	Insects
h	Spiders		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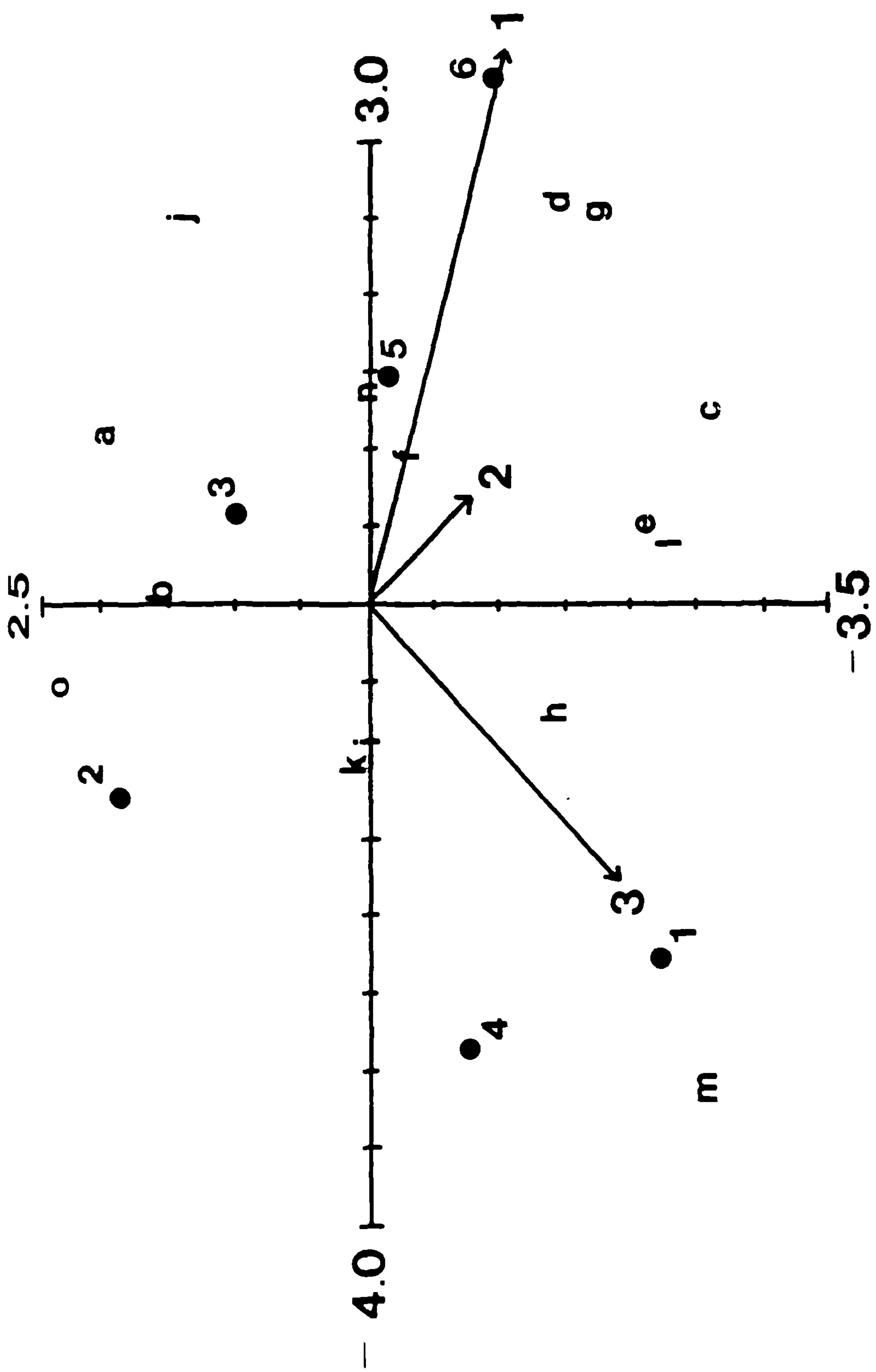
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−4.0

given on the facing pages. Two of the three orientations are shown for each computation.

2. The biplot shows the approximate values of the weighted averages of the species (i.e. weighted by sites) with respect to the environmental variables.

3. The length of the arrow describing an environmental variable is proportional to its influence on the sites *and* species.

4. Species/sites in the same direction from the origin as an arrow are favorably affected by (or at least not inhibited by) that particular variable. Those on the opposite side to the arrow ´dislike´ it. The distance from the origin along the line of an arrow shows the degree to which the particular environmental variable is favoured.

5. All environmental variables are given equal weight in the calculations.

6. Species close to the origin may have their optima within these environmental factors there, or they may be unaffected by the environmental variables chosen. It is possible to determine which by looking at the species by site table in the output (not reproduced here).

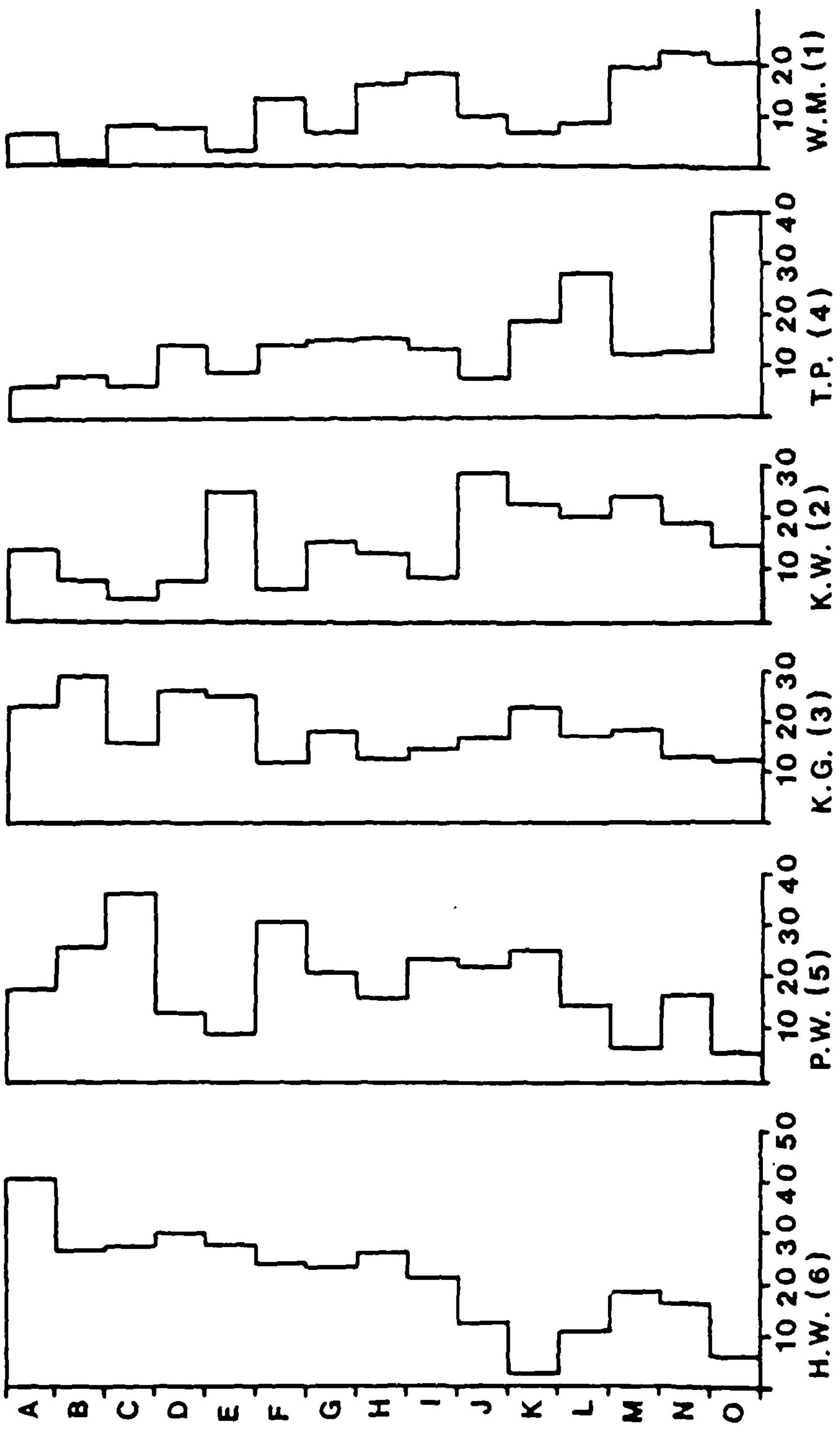
Figure 7.11 shows the results for the grouped species data. Drawn onto this plot is a line following the environmental variable A1 (principal component axis 1) indicating most of the metal effects. Using this line ranks can be assigned to sites and groups according to degree of ´association´ or ´disassociation´ of heavy metal pollution. From this it can be inferred that carabid larvae, pseudoscorpions and molluscs are more numerous in polluted sites while Dermaptera are more prevelant in clean sites. This is also illustrated clearly in Figure 7.12, where the percentage of each group in each site has been plotted using the position along the metal ordination axis, to determine the order of the sites and groups.

How many of the groups are directly affected by the heavy metals can be ascertained by calculating the Pearson product moment correlation coeffecient between environmental variable A1 and each group in turn. Only two groups were significantly correlated, namely Hymenoptera ($r=0.903$, $p<0.05$) and beetle larvae (r0.971, $p<0.01$). Thus it appears that beetle larvae and Hymenoptera favour sites high in metal concentrations or that they are not inhibited from these sites by the level of pollution to the same extent as the other groups studied. Both groups may prove interesting, however pitfall trapping is not an ideal method of trapping these groups, most of the Hymenoptera trapped were bees, wasps and flying parasitic forms rather than ants. As the Hymenoptera consisted of so many flying forms

Figure 7.12 Bar chart to illustrate how the numbers of animals in each group are distributed throughout the sites. Numbers of animals given as percentages of a particular group found at each site. Sites and species groups ordered with respect to PCA axis 1. HW. (site 6) and beetle larvae being associated with high heavy metal concentrations.

Groups of animals:

A Beetle larvae
B Pseudoscorpions
C Molluscs
D Insects
E Millipedes
F Worms
G Beetles
H Hymenoptera
I Centipedes
J Woodlice
K Mammals
L Diptera
M Harvestmen
N Spiders
O Earwigs



A B C D E F G H I J K L M N O
H.W. (6)
10 20 30 40 50
P.W. (5)
10 20 30 40
K.G. (3)
10 20 30
K.W. (2)
10 20 30
T.P. (4)
10 20 30 40
W.M. (1)
10 20

most were not identified to species. Moreover, neither were many of the beetle larvae which are difficult to distinguish, especially below the genus level. Although other groups did not as a whole respond directly to the pollution effect, species within them may show different patterns.

7.5e Discussion of the canonical correspondance analysis on individual groups of animals.

7.5e i. Introduction.

Canonical correspondance analysis was computed for each group of animals for which species determination was made. The resulting biplots for axes 1 and 2 and 1 and 3 are presented for each group. Table 7.9 shows the eigenvalues for each of the three ordination axes in each case. The eigenvalue is always between 0 and 1 and indicates the importance of the ordination axis, the higher the value the greater the importance. The percentage variance accounted for by each axis is also shown to indicate how much variation in the species is due to each axis. It should be noted that although 100% is reached in the third axis of all the plots, some of the variation is due to ´noise´ in the species data and on the whole the eigenvalues are more useful. Table 7.9 also comments on the inter-set correlations of environmental variables with the axes. These are the correlation coefficients between the environmental variables and the species axes consisting of the sample scores, and can give the relative importance of each environmental variable to the final ordination axes.

From the table and the associated biplots, some idea of the relationships between the species and sites and the environmental variables can be gathered. For each species, a considerable amount of information about the particular environmental variables to which it relates can be assembled, as well as its inter-relationships with other species. While much of this information is of particular interest for the biogeography and ecology of the different species, it is of less relevance to the present study. Therefore the following discussion is concerned mostly with topics related to pollution.

By using environmental axis 1 which encompasses the majority of the metal pollution characteristics, those species which are particularly affected positively or negatively by heavy metals can be identified. These are possible indicators of communities in very ´clean´ areas or in polluted areas. Table 7.10 summarises these species for each group of animals studied. For many of the smaller groups, interpretation of the results is relatively straight forward, and so the discussion of the results concentrates on these groups first before continuing to the more complex plots. Pearson product moment correlations have been used to clarify some of the relationships. All environmental

Table 7.9

DETAILS OF CCA OUT FILES

DATA SET	EIGENVALUES			CUMMULATIVE % ACCOUNTED FOR			COMMENTS ON INTER-SET CORRELATIONS*		
	Axes 1	2	3	1	2	3	1	2	3
Grouped	0.046	0.028	0.013	52.4	85.0	100	1	2	3
Millipedes	0.541	0.227	0.128	60.4	85.7	100	1	3	2
Woodlice	0.237	0.107	0.001	68.8	99.9	100	2,3	1	
Molluscs	0.131	0.083	0.062	47.4	77.4	100	1	2	3
Worms	0.174	0.114	0.074	47.2	79.7	100	1	2	3
Harvestmen	0.421	0.106	0.021	76.9	96.2	100	2	1,2	1
Spiders	0.444	0.229	0.108	56.9	86.2	100	1	1,2,3	2,3
Carabids	0.310	0.160	0.125	52.1	79.0	100	1	2	3
Centipedes	0.131	0.053	0.041	58.2	81.8	100	1	2	3
Pooled	0.335	0.229	0.105	50.1	84.2	100	1	1,2	3

Table 7.9 contd

* Indicates the environmental variable most influential in each ordination axis. As each environmental variable was an axis from the PCA calculation, the number in each column represents the appropriate PCA axis. Thus 1 = PCA axis 1 which was mostly metal concentrations, 2 = PCA axis 2 mostly lead and copper in the soil, 3 = PCA primarily soil type and density of trees. Two numbers indicate that both axes were influential.

Table 7.10

SPECIES INDICATIVE OF CLEAN OR CONTAMINATED AREAS, AS DETERMINED USING THE CCA BIPLOTS

Group	'Clean Species'	'Contaminated Species'
Millipedes	*Nemasoma varicorne*	*Chordeuma proximum*
	Ophyiulus pilosus	*Glomeris marginata*
	Tachypodioulus niger	*Polydesmus angustus*
	Polydesmus gallicus	
Woodlice	*Philoscia muscorum*	*Haplophthalmus danicus*
	Armadillidium vulgare	
Molluscs	*Limax marginatus*	*Punctum pygmaeum*
	Deroceras reticulatum	*Arion subfuscus*
	Cochlicopa lubricella	*Oxychilus celarius*
Harvestmen	*Mitostoma chrysomelas*	*Nemastoma bimaculatum*
	Anelasmocephalus cambridgii	*Leiobunum rotundum*
		Leiobunum blackwallii
Spiders	*Coelotes atropos*	{Genus *Pardosa*}
	Agroeca brunnea	
	Anyphaena accentuata	
	Centromerus sylvaticus	
	Stemonyphantes lineatus	
Carabids	*Agonum dorsale*	*Cychrus caraboides*
	Agonum obscurum	*Carabus nemoralis*
	Agonum viduum	
	Pterostichus cupreus	*Metabletus foveatus*
	Carabus granulatus	*Leistus fulvibarbis*
	Leistus rufomarginatus	*Agonum albipes*
	Bembidion tetracolum	
Centipedes	*Strigamia accuminata*	*Strigamia crassipes*
	Lithobius crassipes	

variables were log transformed before such calculations were undertaken.

7.5e ii. Millipedes. (Figures 7.13a and b).

The millipede fauna is notable for the fact that one species, Chordeuma proximum, was found only at the most polluted site 6 (HW) where it occurred in large numbers. It is not surprising that this species appears on the biplots a long way along environmental variable 1, corresponding to most of the metal effects. It´s abundance was also positively correlated to this axis ($r=0.903$, $p<0.05$) and to many of the different types of metal concentrations measured. Also showing similar correlations is Glomeris marginata which was found at all sites except 4 (TP); this may be a rather better species characterising polluted areas as it is more widespread (except that Hopkin et al. 1985 recorded its absence from Hallen wood, a polluted wood close to HW, site 6). The positive correlation of this species with litter depth ($r=0.929$, $p<0.01$) may also highlight an important feature of this animal´s preferred habitat (litter depth is not necessarily positively correlated with metal concentrations; only cadmium and zinc in the litter layer gave significant correlations with litter depth $r=0.813, 0.873$, $p<0.05$). The only other species found close to the high pollution end of the plot is Polydesmus angustus. This is a very wide ranging species recorded by Blower (1985) as often occurring in situations more acidic

Figure 7.13 CCA of millipedes.

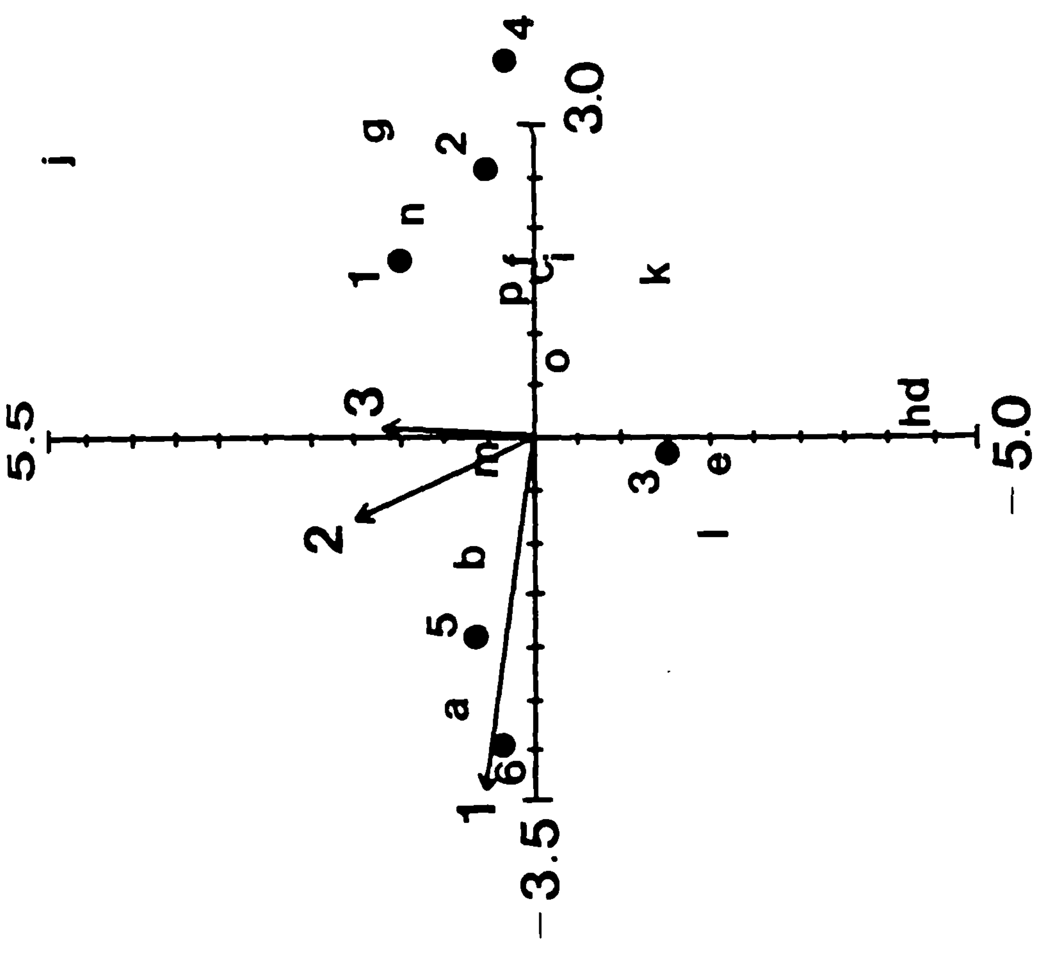
7.13a Axis 1 horizontal, axis 2 vertical.
7.13b Axis 1 horizontal, axis 3 vertical.

Sites as in Figure 7.7

Environmental variables:
1 PCA axis 1
2 PCA axis 2
3 PCA axis 3

Species:

a *Chordeuma proximum*
b *Glomeris marginata*
c *Tachypodoiulus niger*
d *Ommatoiulus sabulosus*
e *Julus scandinavius*
f *Ophyiulus pilosus*
g *Brachyiulus pusillus*
h *Cylindroiulus britannicus*
i *Cylindroiulus punctatus*
j *Nemasoma varicorne*
k *Proteroiulus fuscus*
l *Polydesmus angustus*
m *Polydesmus denticulatus*
n *Polydesmus gallicus*
o *Brachydesmus superus*
p *Nanogona polydesmoides*


5.5
−3.5
3.0
−5.0
1
2
3
4
5
6
a
b
e
g
hd
i
j
k
l
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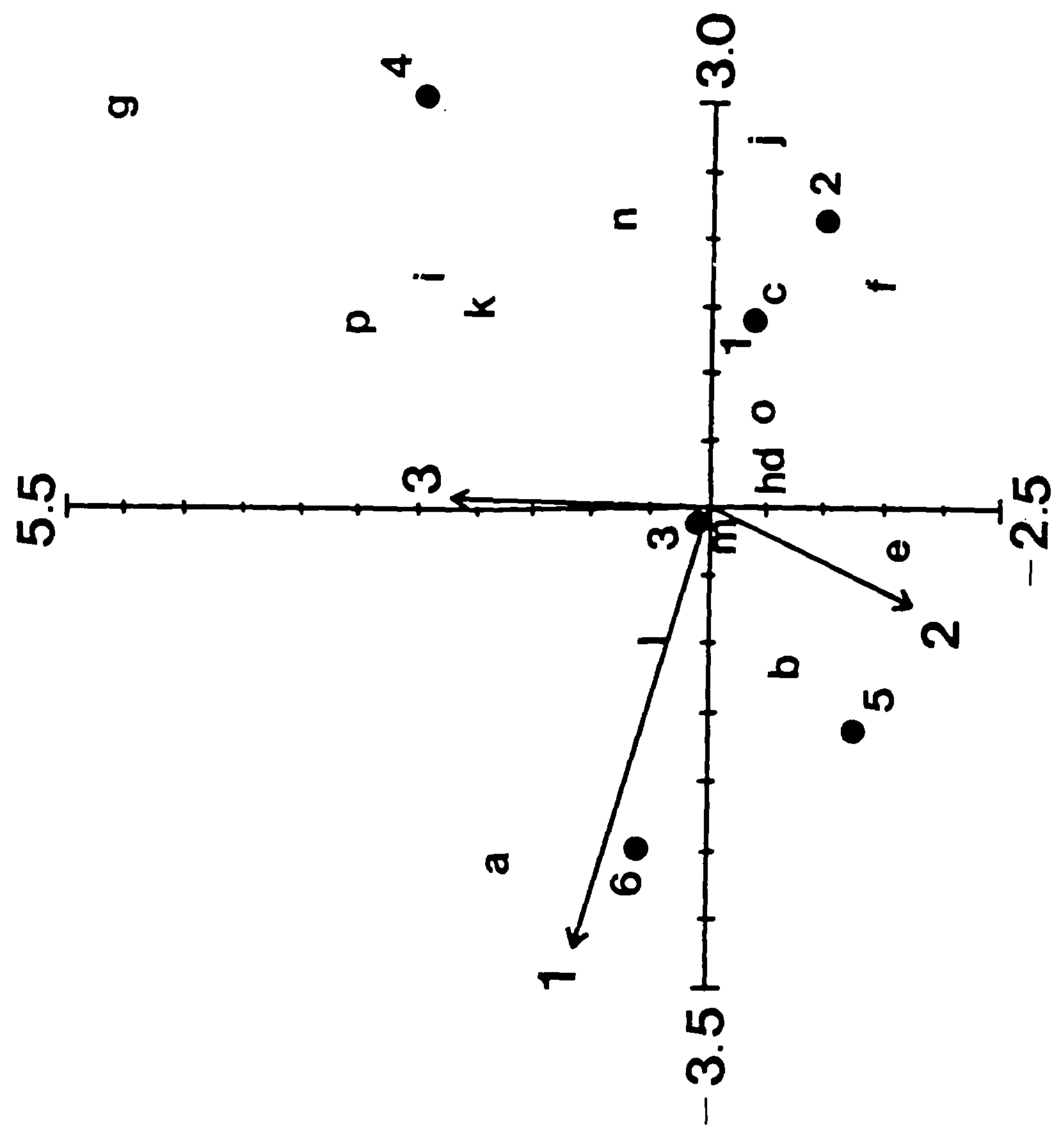
n
o
p
f
c


5.5
-2.5
-3.5
3.0
1
2
3
4
5
6
a
b
c
e
f
g
hd
i
j
k
l
m
n
o
p

than most millipedes; indeed, it shows a significant negative correlation with pH ($r=-0.831$, $p<0.05$) in this study.

Nearly all the remaining millipede species occur on the other side of the biplot from environmental axis 1, although very few record significant negative correlations with any metal concentrations. Both Tachypodoiulus niger and Ophyiulus pilosus are found away from effects of the pollution, this is perhaps surprising since both are considered to be good colonisers (Blower 1969) and might be expected to be more adaptable than some other species.

The eigenvalue for the first ordination axis is 0.541 which is the highest value among the taxonomic groups. By referring to the inter-set correlations it is confirmed that the first environmental variable (ie. that representing the majority of the metal-related factors) makes the largest contribution to the first ordination axis.

In conclusion, it would appear that while most species of millipede are adversely affected by heavy metal pollution, a few species are able to survive the levels found at Haw wood and are able to thrive there.

7.5e iii. Woodlice. (Figures 7.14a and b).

The eigenvalue for the woodlice CCA is 0.237; this is lower than that for the millipedes and implies that the first ordination axis is not as important as that of the millipedes in explaining the variation. Also, in contrast to the millipedes it is the second and third environmental variables which have the greatest influence on the first ordination axis. The metals (*environmental axis 1*) primarily influence the *second ordination axis*. This can also be seen by the position of the arrows in Figure 7.14a.

One species is associated with high pollution, Haplophthalmus danicus, and two prefer clean areas Philoscia muscorum and Armadillidium vulgare; none of these species were correlated with environmental variable 1. The first of these species (H. danicus) was found in low numbers and it is a surprising species to be found in this situation. Oniscus asellus, the most widespread species in the present study, occupies a central position and is not greatly influenced in either direction. Again, those species renowned for their colonising ability, e.g. A. vulgare (Harding & Sutton 1985) have proved to be less resistant or adaptable to pollution.

Figure 7.14 CCA of woodlice.

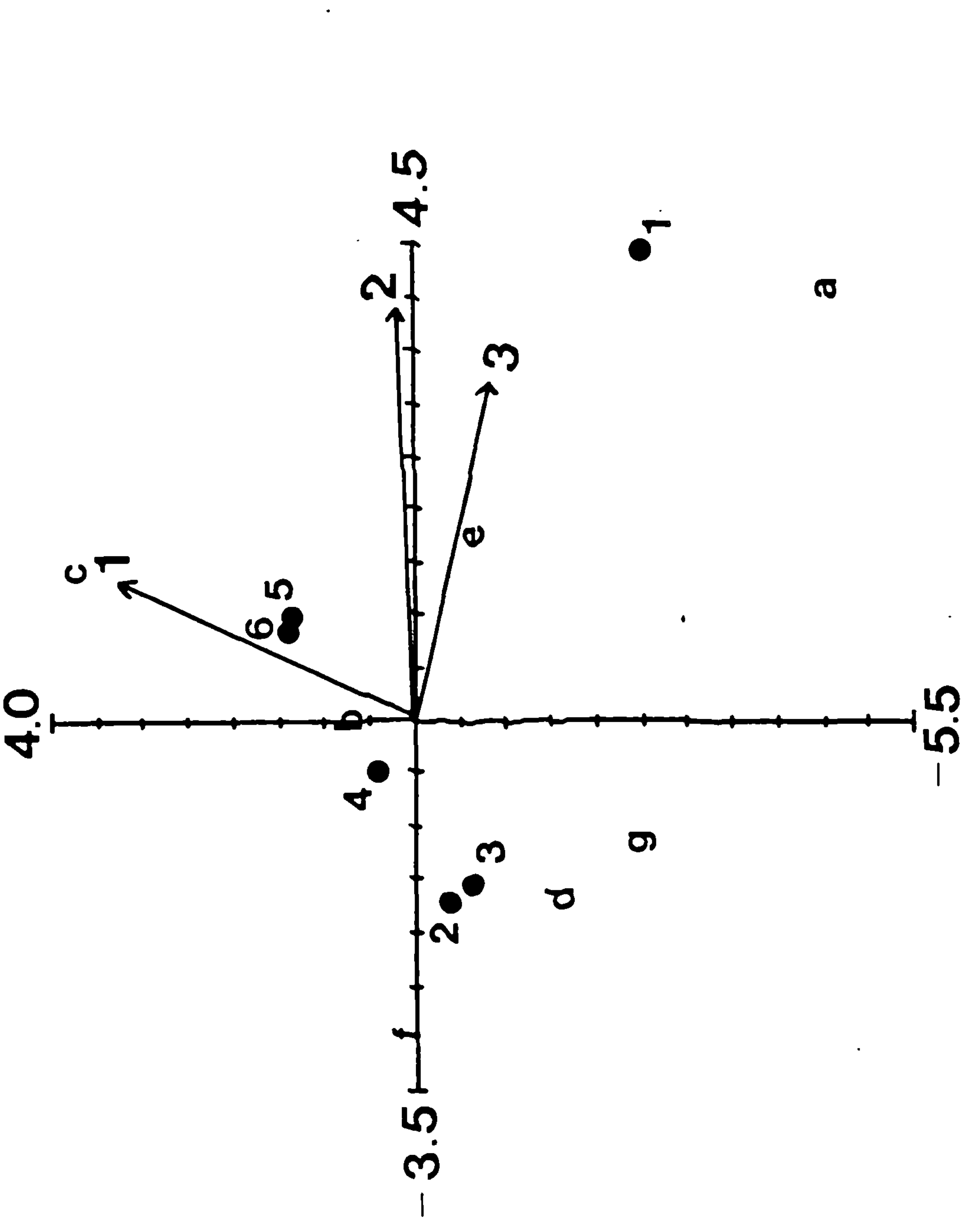
7.14a Axis 1 horizontal, axis 2 vertical.
7.14b Axis 1 horizontal, axis 3 vertical.

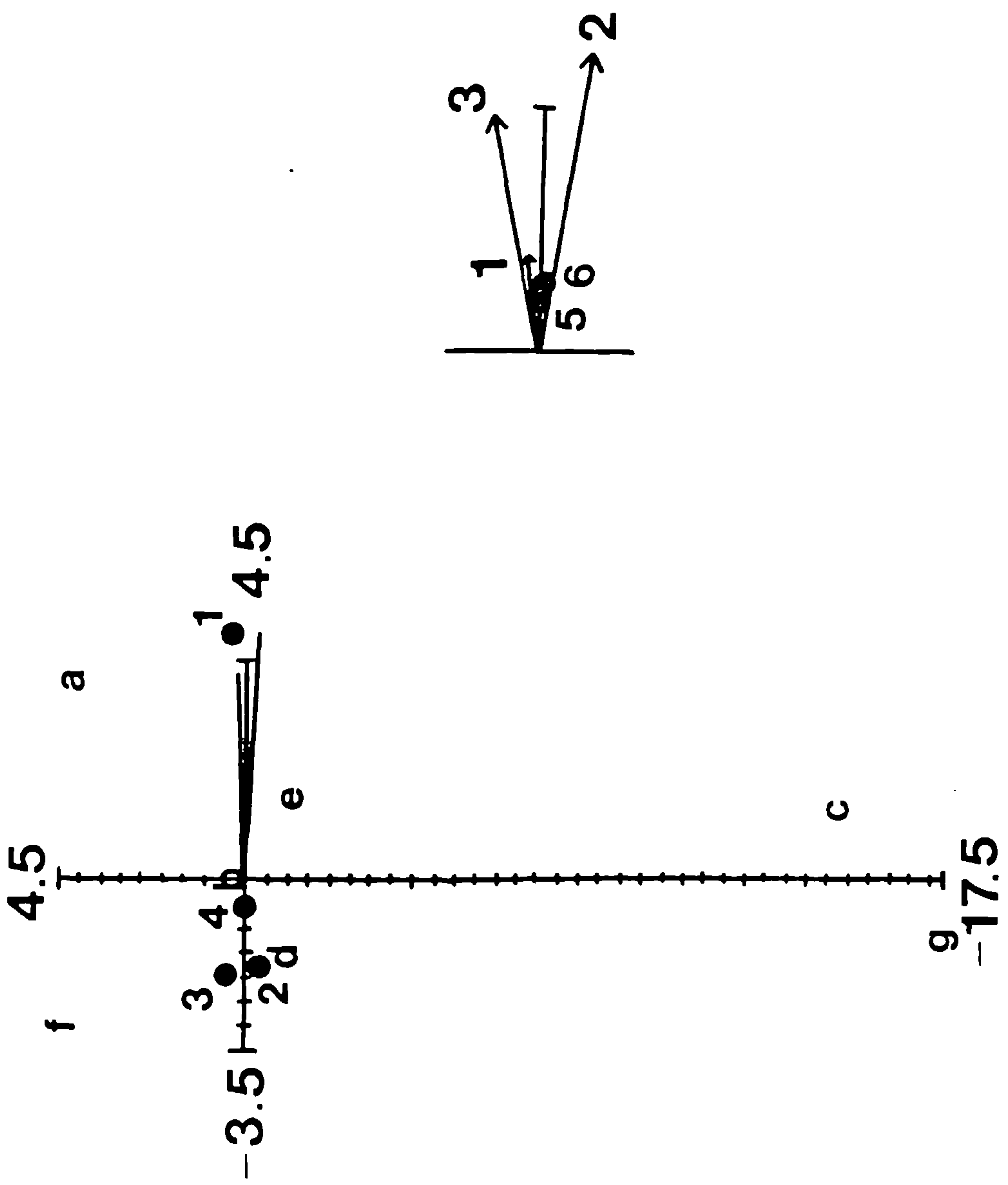
Sites as in Figure 7.7

Environmental variables:
1 PCA axis 1
2 PCA axis 2
3 PCA axis 3

Species:

a *Ligidium hypnorum*
b *Oniscus asellus*
c *Haplophthalmus danicus*
d *Philoscia muscorum*
e *Trichoniscus pusillus*
f *Porcellio scaber*
g *Armadillidium vulgare*


4.0
-3.5
4.5
-5.5
a
b
c
d
e
f
g
1
2
3
4
5
6


4.5
-3.5
4.5
-17.5
a
b
c
d
e
f
g
1
2
3
4
5
6

7.5e iv. Worms. (Figures 7.15a and b).

The biplots for the worm data are rather easier to interpret. Environmental variable 1 (metal concentrations) is most influential in ordination axis 1, variable 2 (lead and copper in the soil) in axis 2 and variable 3 (tree density and soil type) in axis 3. Thus the effect of the metals is most important in the first ordination axis. Three species are found where the heavy metal pollution is highest, namely *Allolobophora callaginosa*, *Lumbricus terrestris* and *Octolasium cyaneum*, and two where it is lowest, namely *Dendrobaena rubida* and *Eisnia foetida*. Pitfall trapping is not a well recognised method of catching worms, and will only capture surface-active animals. *A. callaginosa* and *O. cyaneum* do occur within the top 8cm of the soil surface particularly when immature (Edwards & Lofty 1977), but *L. terrestris* is a much deeper dwelling animal. All these three species are represented in the collection by low numbers of animals and most are probably chance captures, although it is interesting that they were present at Haw wood at all. It is probably wise not to place to much emphasis on these results owing to the low numbers of animals of all species caught.

Figure 7.15 CCA on worms.

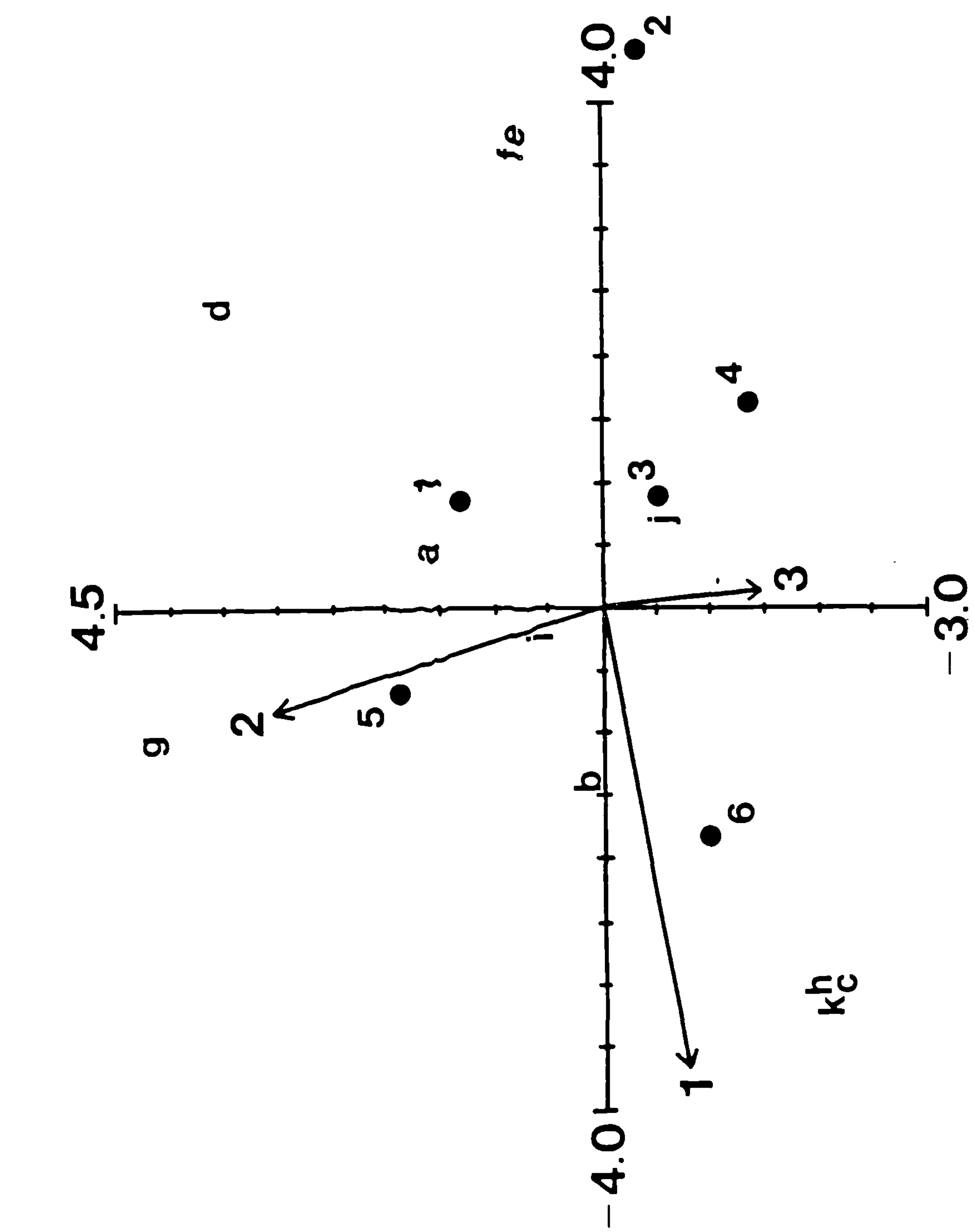
7.15a Axis 1 horizontal, axis 2 vertical.
7.15b Axis 1 horizontal, axis 3 vertical.

Sites as in Figure 7.7

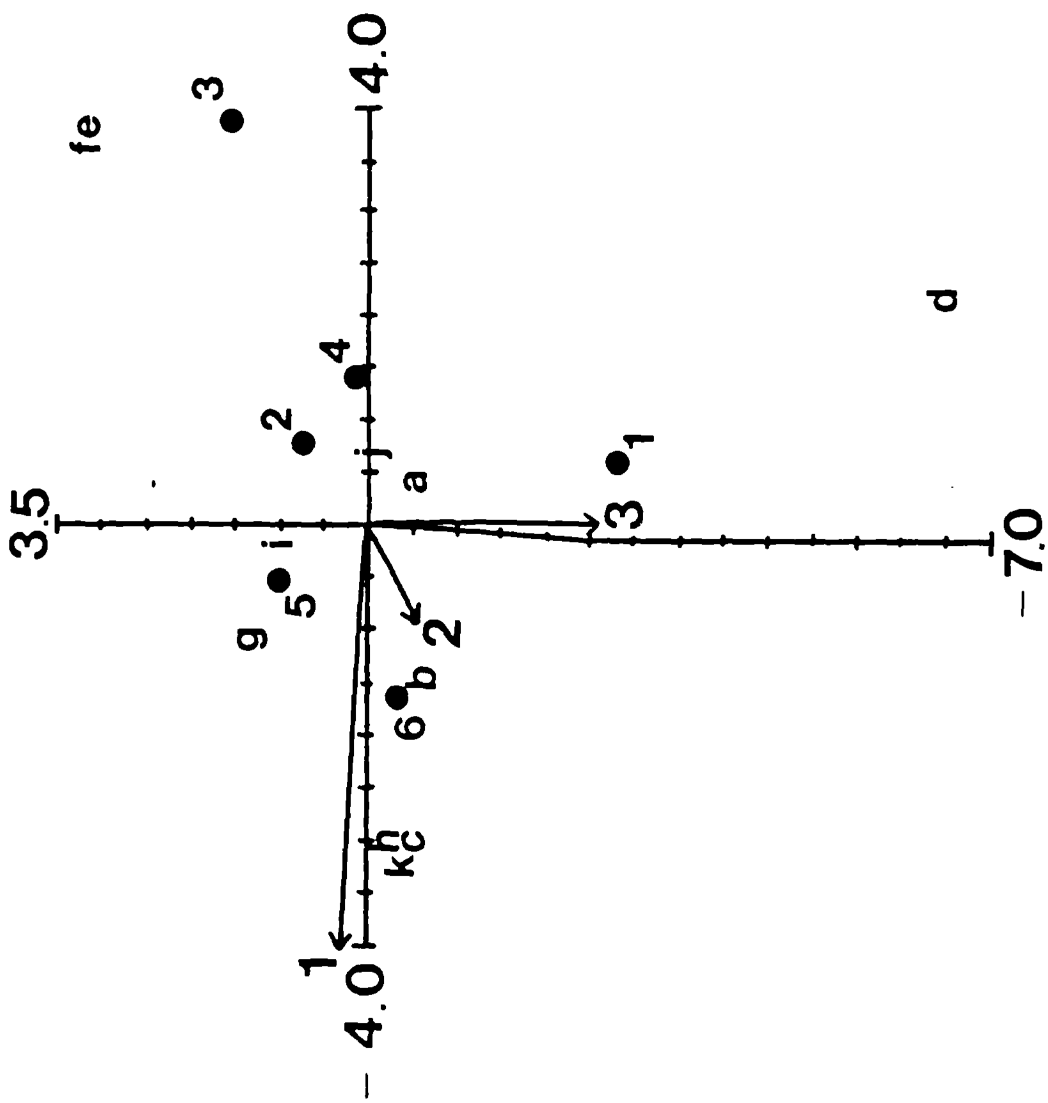
Environmental variables:
1 PCA axis 1
2 PCA axis 2
3 PCA axis 3

Species:

a *Aporrectodea caliginosa*
b *Ap. rosea*
c *Allolobophora chlorotica*
d *Eiseniella tetraedra*
e *Eisenia foetida*
f *Dendrobaena rubidus*
g *Dendrobaena octaedra*
h *Lumbricus terrestris*
i *L. rubellus*
j *L. castaneus*
k *Octolasion cyaneum*



4.5
−3.0
4.0
−4.0


3.5
4.0
–7.0
– 4.0
fe
d
a
j
i
g
b
h
k
c
1
2
3
4
5
6

7.5e v. Molluscs. (Figures 7.16a and b).

Among the molluscs *Cochlicopa lubricella*, *Limax marginatus* and *Deroceras reticulatum* . appear to be the species found in polluted areas; these and several other species are positively correlated with various metal concentrations. A number of molluscs species appear to ´prefer´ unpolluted areas. Snails are not renowned for high levels of activity and although 30 species were recorded in all, most were found in low numbers, usually under five individuals per site caught throughout the whole trapping time. The principal exceptions to this was site 5 (PW) which was particularly rich in molluscs and also two species of slug which were caught in larger numbers from several sites. Of the two slug species one, *Arion sylvaticum* was abundant in several sites and is represented on the biplot in a central position. A more detailed survey of the molluscs may be worth while, but a better method of collection needs to be found.

7.5e vi. Centipedes. (Figures 7.17a and b).

The metal-related environmental variable has most influence on the first ordination axis, and the influence of this on the animals is large since the corresponding arrow is very long. *Strigamia accuminata* and *Lithobius crassipes* are associated with the polluted sites; the former is

Figure 7.16 CCA of molluscs.

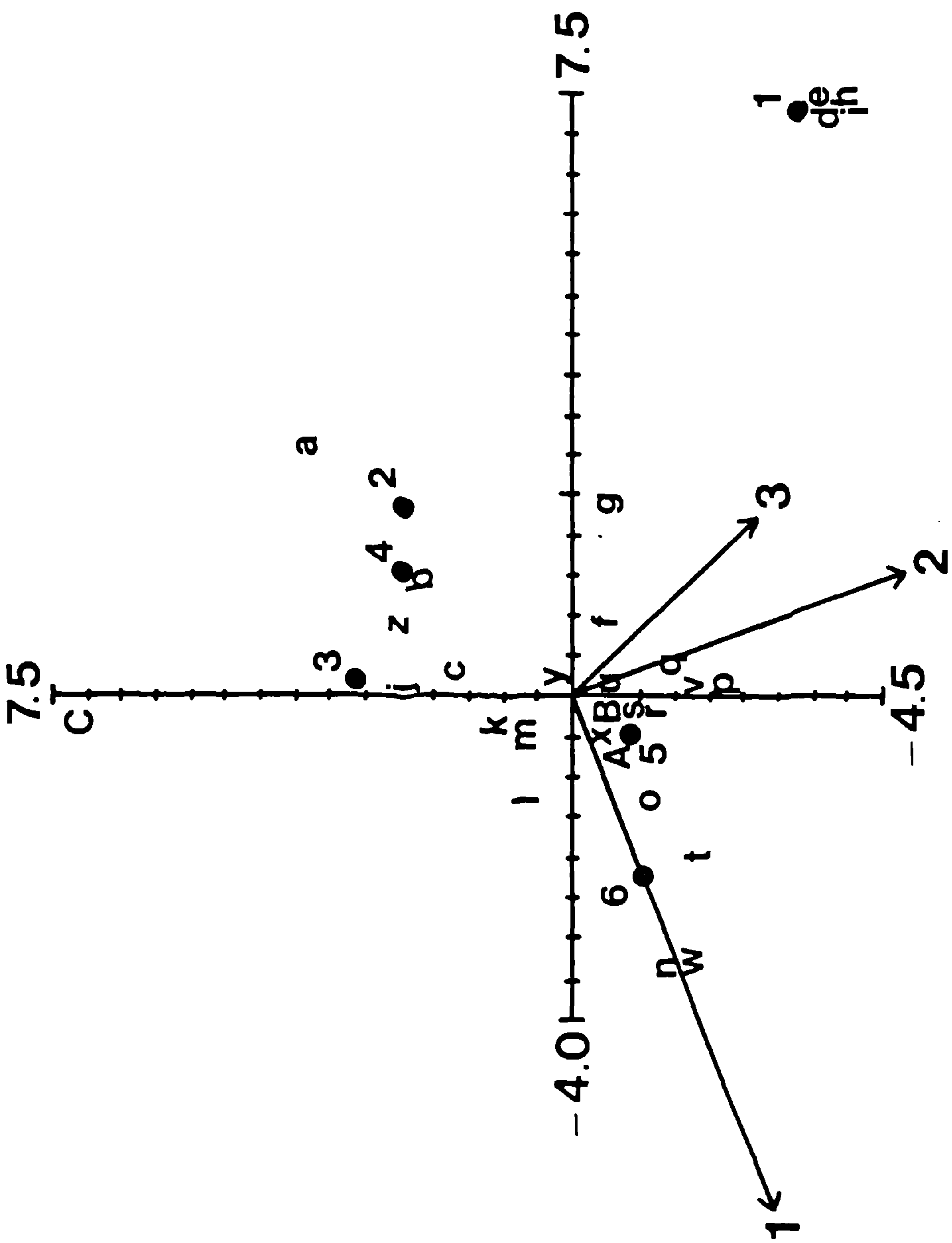
7.16a Axis 1 horizontal, axis 2 vertical.
7.16b Axis 1 horizontal, axis 3 vertical.

Sites as in Figure 7.7

Environmental variables:
1 PCA axis 1
2 PCA axis 2
3 PCA axis 3

Species:

a *Punctum pygmaeum*
b *Retinella niditula*
c *Vitrina pellucida*
d *Oxychilus cellerarius*
e *O. draparnaudi*
f *O. helveticus*
g *Helicella virginiana*
h *Columella edentula*
i *Monacha cartusiana*
j *Euconulus fulvus*
k *Vitrea crystallina*
l *Marpessa laminata*
m *Discus rotundatus*
n *Limax marginatus*
o *Cepaea nemoralis*
p *Retinella radiata*
q *Clausilia bidentata*
r *Trichia hispida*
s *Zonitoides nitidus*
t *Cochlicopa lubricella*
u *Cepaea hortensis*
v *Acanthinula aculeata*
w *Derocerus reticulatum*
x *Arion hortensis*
y *A. sylvaticus*
z *A. subfuscus*
A *Milax sowerbyi*
B *Arion ater*
C *Limax maximus*


7.5
7.5
-4.0
-4.5

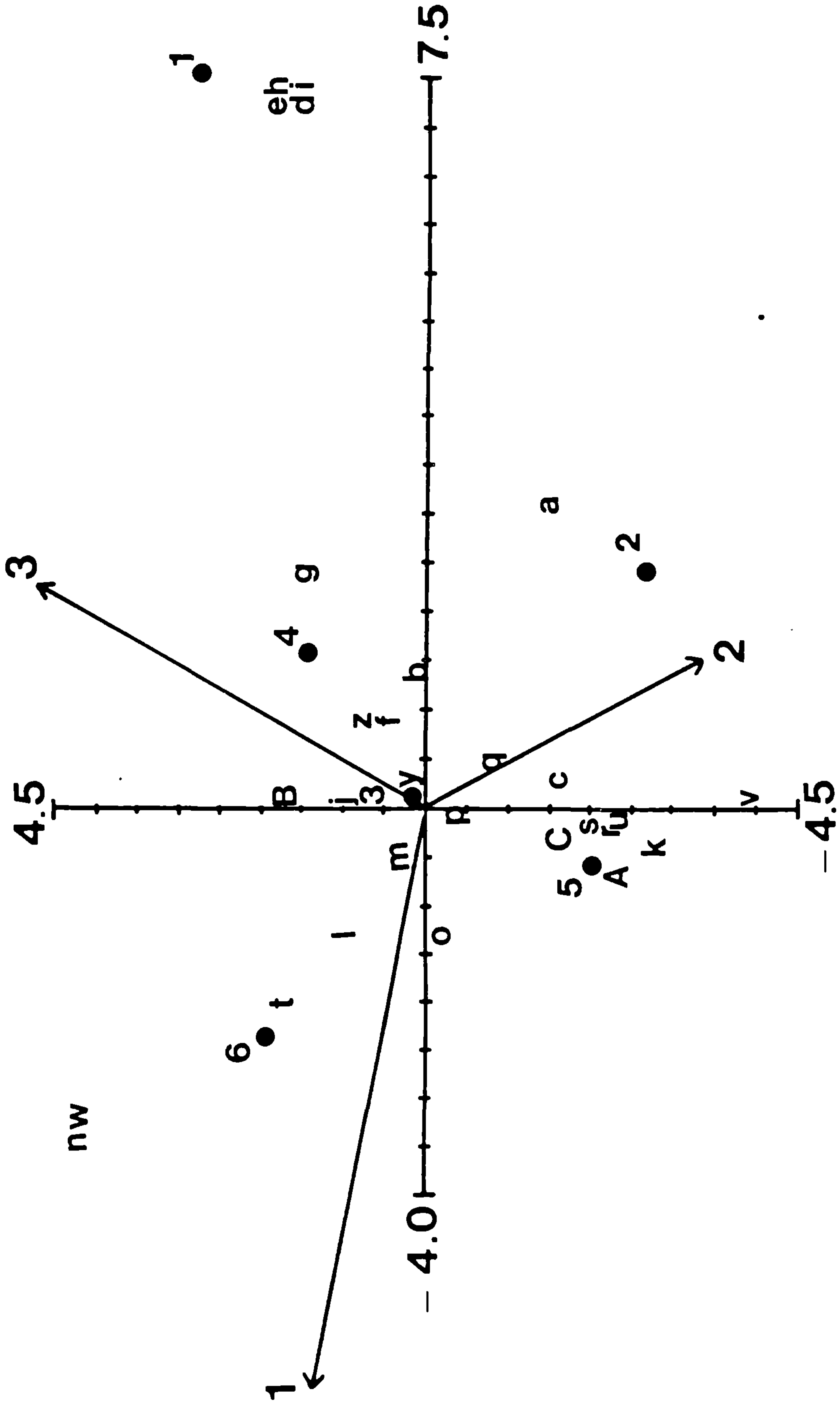

4.5
−4.5
7.5
−4.0
nw

Figure 7.17 CCA of centipedes.

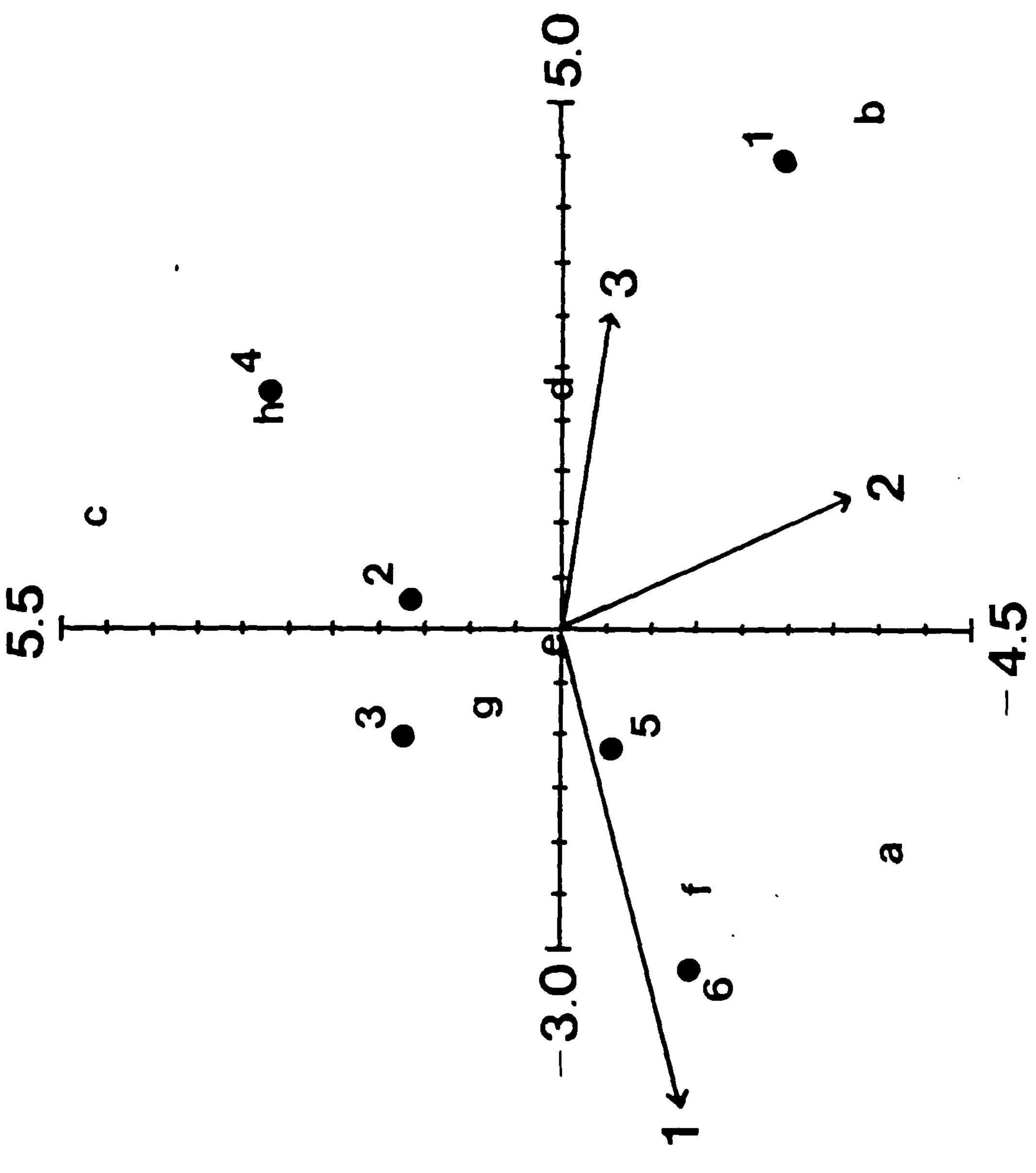
7.17a Axis 1 horizontal, axis 2 vertical.
7.17b Axis 1 horizontal, axis 3 vertical.

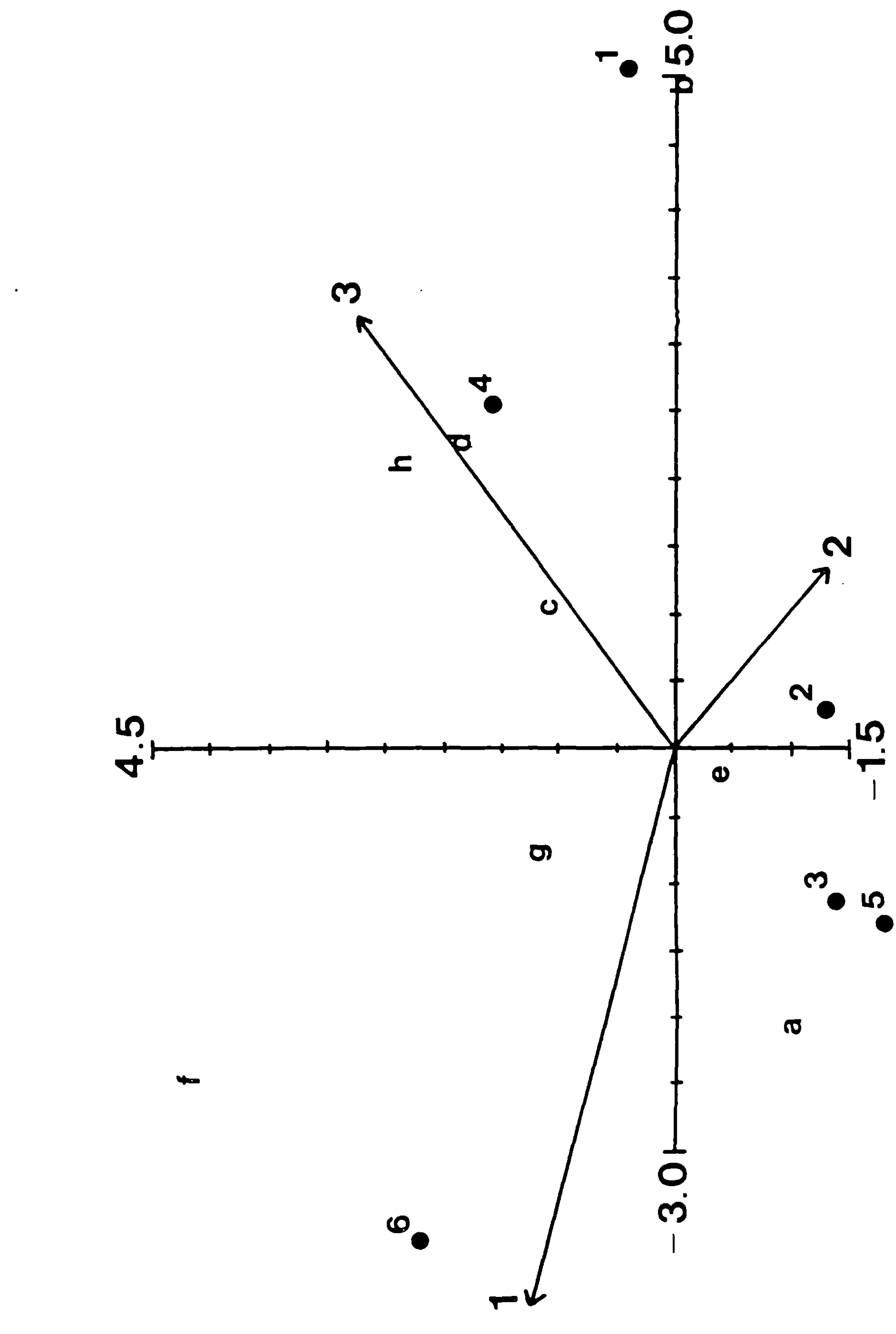
Sites as in Figure 7.7

Environmental variables:
1 PCA axis 1
2 PCA axis 2
3 PCA axis 3

Species:

a *Strigamia acuminata*
b *Strigamia crassipes*
c *Brachygeophilus truncorum*
d *Lithobius forficatus*
e *Lithobius variegatus*
f *L. crassipes*
g *L. microps*
h *L. borealis*


5.5
5.0
−4.5
−3.0
1
2
3
a
b
c
d
e
f
g
h
1
2
3
4
5
6



4.5
−1.5
5.0
−3.0
1
2
3
a
b
c
d
e
f
g
h
1
2
3
4
5
6

significantly correlated with environmental variable 1 ($r=0.898$, $p<0.05$) and the latter with all the metal concentrations in all the soil layers ($r>0.811$, $p<0.05$). *Strigamia crassipes* is related to clean sites. It is interesting to note here the difference in behaviour of the two species of *Strigamia*. They are widely separated with regard to metal concentrations but close in terms of environmental variables 2 and 3. Again the common species *Lithobius variegatus* occupies a central position.

7.5e vii. *Harvestmen*. (Figures 7.18a and b).

The main metal-related environmental variable in the harvestmen calculations contributes to ordination axes 2 and 3 but not to axis 1. The second environmental variable is more important in the first ordination axis. The species preferring polluted areas are the two *Leiobunum* species, *L. blackwalli* and *L. rotundum* and *Nemastoma bimaculatum*. The first of these (*L. blackwalli*) is positively correlated with environmental variable 1 ($r=0.94$, $p<0.05$) and also with nearly all the metal concentrations in the soil layers ($r>0.851$, $p<0.05$). Those species found in non-polluted areas are principally *Mitostoma crysomelas* (occurring at site 1 (WM) only) and *Anelasmocephalus cambridgii*, which was also found in the most polluted site (HW, site 6) in very low numbers. This last species is interesting as it was listed as a possible indicator species from the log normal plots (section 7.2) because it was believed to increase in

Figure 7.18 CCA of harvestmen.

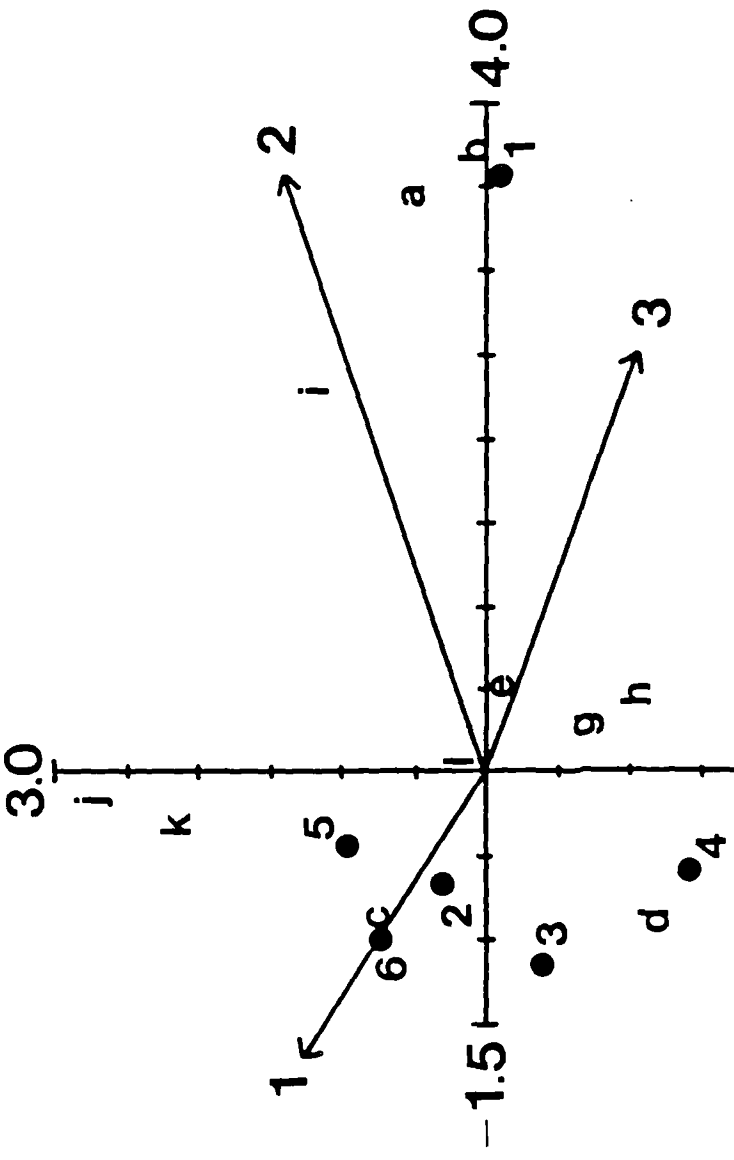
7.18a Axis 1 horizontal, axis 2 vertical.
7.18b Axis 1 horizontal, axis 3 vertical.

Sites as in Figure 7.7

Environmental variables:
1 PCA axis 1
2 PCA axis 2
3 PCA axis 3

Species:

a *Anelasmocephalus cambridgii*
b *Mitostma crysomelas*
c *Nemastomabimaculatum*
d *Paroligolophus agrestis*
e *Phalangium opilio*
f *Lophopilio palpinalis*
g *Oligolophus tridens*
h *Lacinius ephippiatus*
i *Mitopus morio*
j *Leiobunum blackwalli*
k *L. rotundum*
l *Rilaena triangularis*


3.0
4.0
−1.5
−3.5
1
2
3
a
b
c
d
e
f
g
h
i
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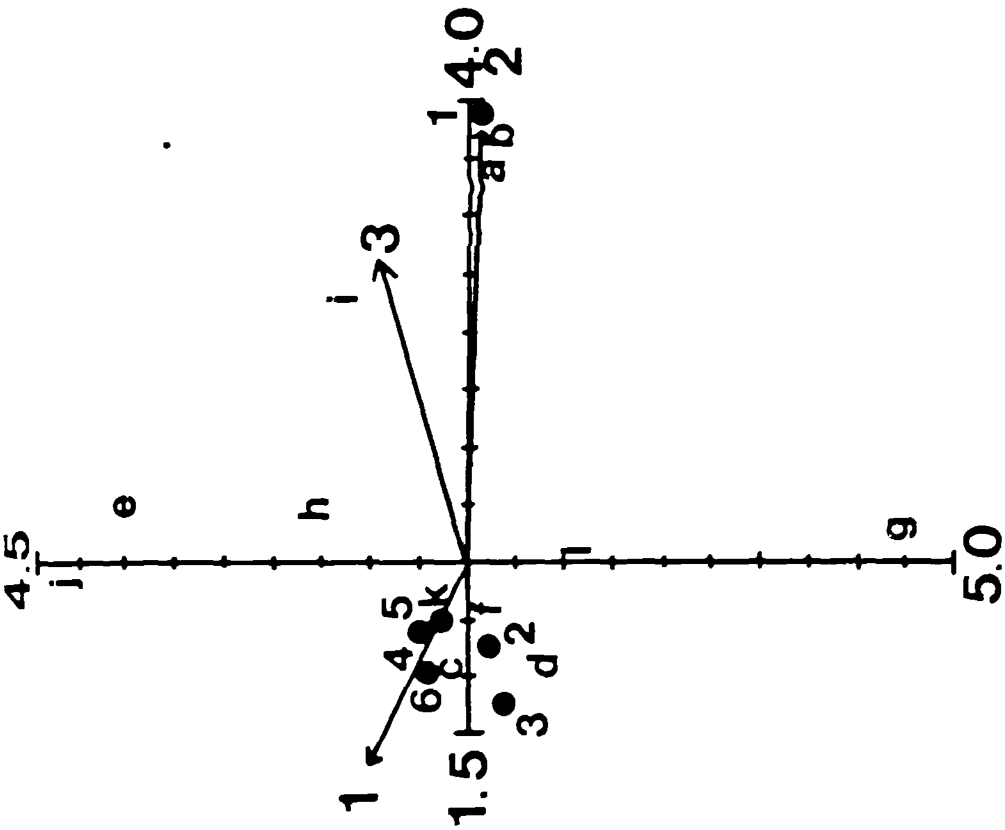
2
3
4
5
6



4.5
5.0
1.5
4.0

numbers with low levels of pollution. Despite its position on the biplots it was found in high numbers at site 5 (PW). Another indicator species suggested from the log normal plots was Rilaena triangularis. In the biplots this widespread species occupies a central position.

N. bimaculatum is unusual among common British harvestman species because it does not complete its life cycle in one year (Williams 1962). Most other harvestmen are annuals and over winter as eggs (except R. triangularis which over winters as immatures, Todd 1949). Species with shorter life cycles might adapt more easily to situations with high pollution, but N. bimaculatum has managed to overcome the problem of high metal concentrations and has a longer life cycle than most harvestmen.

7.5e viii. Carabid beetles. (Figures 7.19a and b).

CCA for the carabids results in a first ordination axis which is very close to the environmental variable 1, the metal effect. This is shown in the inter-set correlations and the biplots. Less than half of the species recorded are situated on the polluted side of the biplots and those particularly associated with high levels of pollution are: Cychrus caraboides, Carabus nemoralis, Carabus violaceus, Metabletus foveatus and Agonum albipes. All of these species are positively correlated with environmental variable 1 and the majority of the individual metal

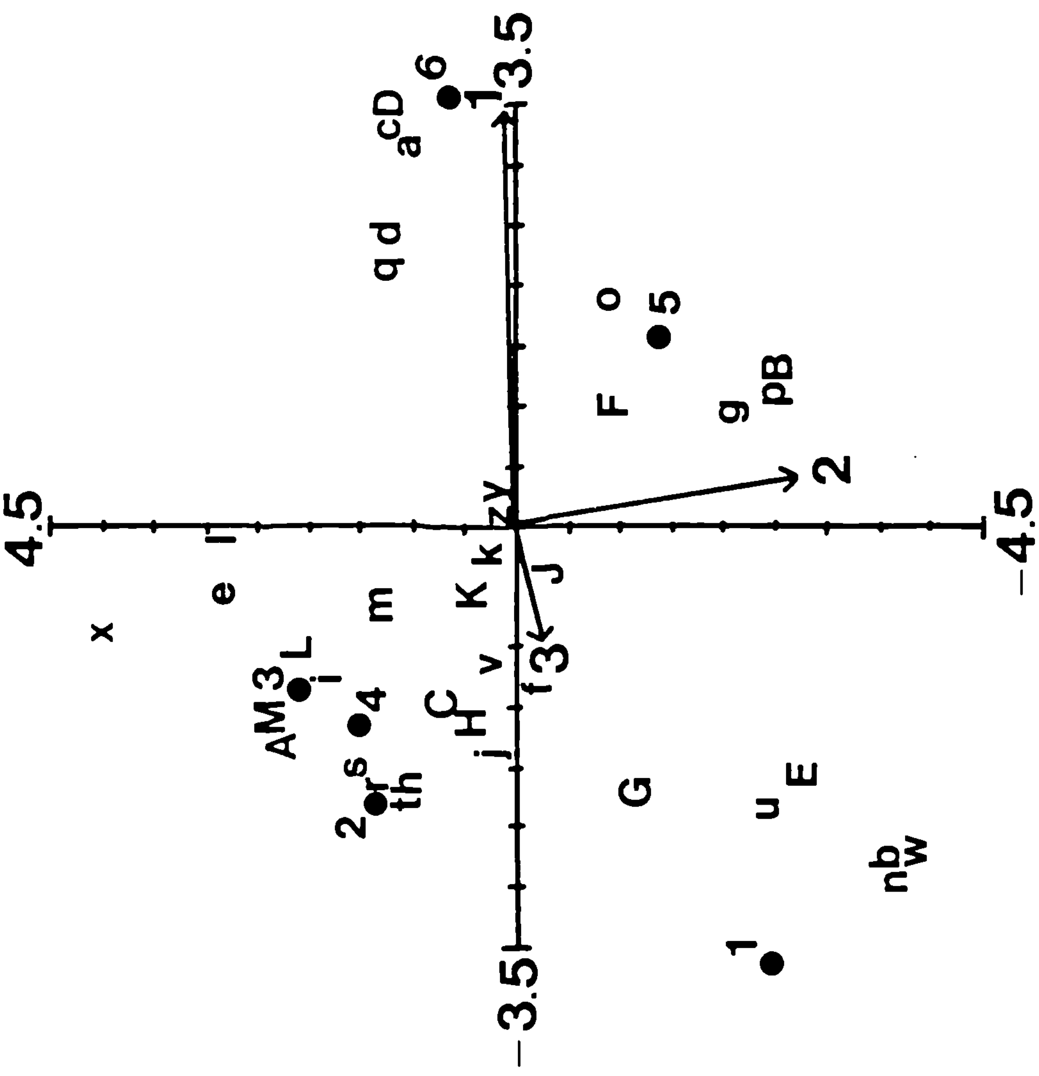
Figure 7.19 CCA of carabids.

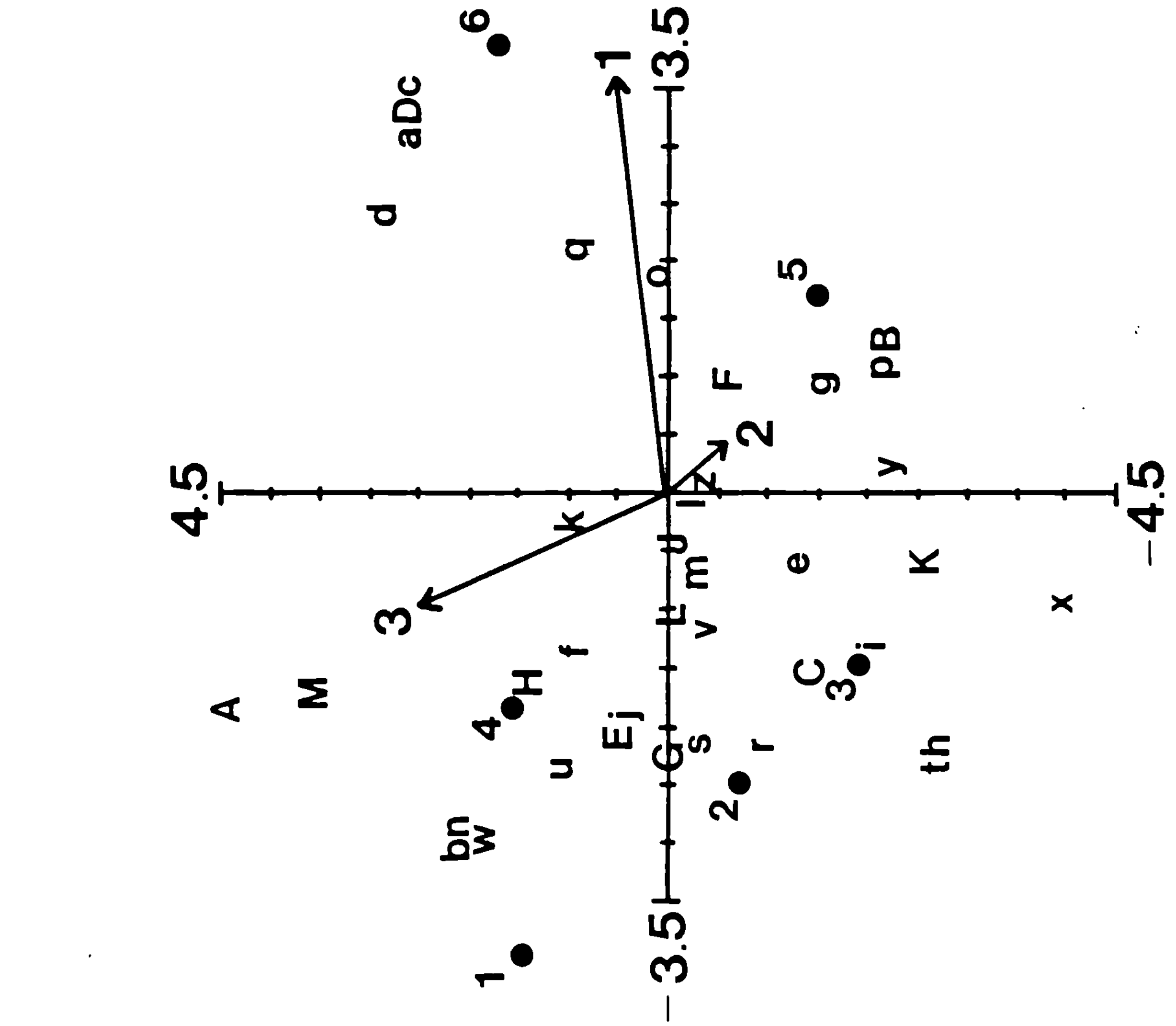
7.19a Axis 1 horizontal, axis 2 vertical.
7.19b Axis 1 horizontal, axis 3 vertical.

Sites as in Figure 7.7

Environmental variables:
1 PCA axis 1
2 PCA axis 2
3 PCA axis 3

Species:
a *Cychrus caraboides*
b *Carabus granulatus*
c *C. nemoralis*
d *C. violaceus*
e *Loricera pilicornis*
f *Pterostichus madidus*
g *P. niger*
h *P. nigrita*
i *P. melanarius*
j *P. cupreus*
k *P. strenuus*
l *P. versicolor*
m *Leistus ferrugeneus*
n *L. rufomarginatus*
o *L. fulvibarbis*
p *Clivina fossor*
q *Agonum albipes*
r *A. dorsale*
s *A. obscurum*
t *Agonum viduum*
u *A. assimile*
v *Bembidion guttula*
w *B. tetracolum*
x *Calathus piceus*
y *C. melanocephalus*
z *Abax parallelepipedus*
A *Dromius linearis*
B *Bradycellus harpalinus*
C *Platyderis ruficolis*
D *Metabletus foveatus*
E *Asaphidion flavipes*
F *Nebria brevicollis*
G *Notiophilus rufipes*
H *N. biguttatus*
J *Dromius quadromaculata*
K *Amara familiaris*
L *Amara similata*
M *Harpalus rufipes*


4.5
-4.5
3.5
-3.5



concentrations (r>0.811, p<0.05). A far larger number of species are related to low levels of pollution: *Carabus granulatus*, *Leistus rufomarginatus*, *Bembidion tetracolon*, *Agonum viduum*, *A. obscurum*, *Pterostichus nigrita* and several more can be identified in this position. It should again be noticed that different species in the same genus respond differently to the environmental variables including the pollution effect, in particular the genera *Leistus* and *Agonum*. Because of the larger numbers of species on the plots and the spread of the sites (in comparison to earlier plots) it is possible to identify a particular carabid fauna (or components of it) at sites 5 (PW) and 6 (HW) and to a lesser extent site 1 (WM), whereas sites 2 (KW), 3 (KG) and 4 (TP) are more similar.

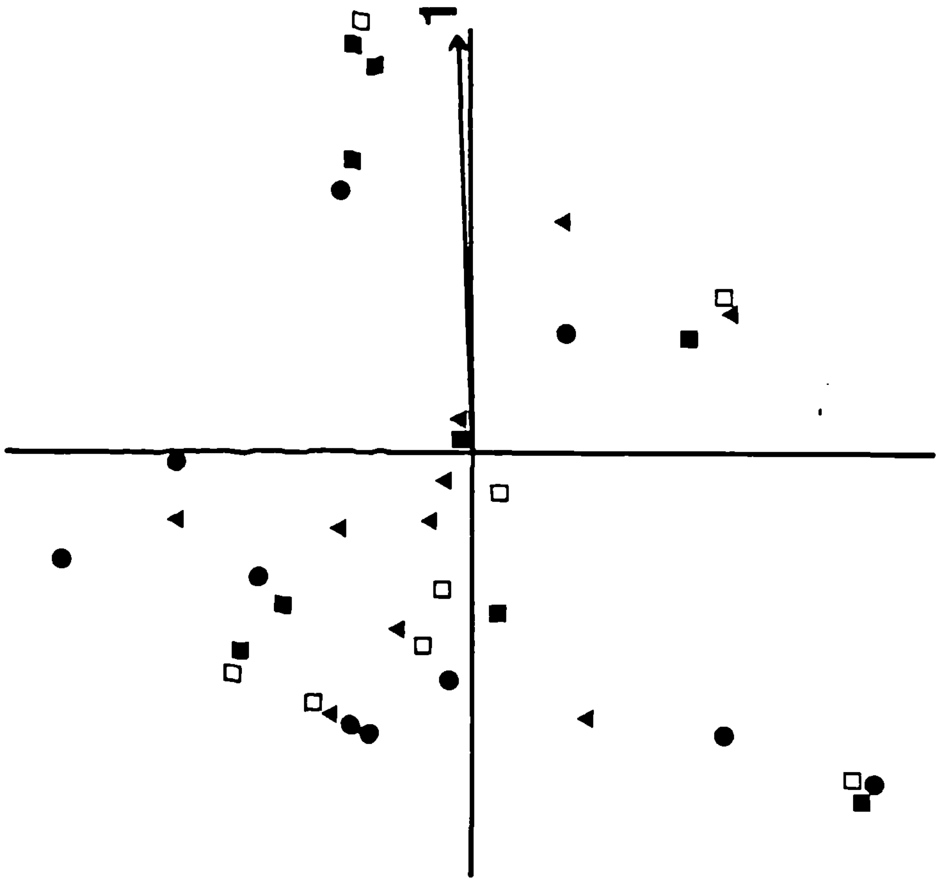
One notable feature of the species found at the polluted site 6 (HW) is their larger body size in comparison to those found at ´clean´ sites. These were not accidental captures as some were caught in large numbers (for example *Carabus violaceus* and *Cychrus caraboides*). The body size of beetles was an aspect considered by Read *et al*. (1987), who found no correlation between the median size of animals at the sites and metal concentrations although the sites contained different proportions of individuals belonging to the size groups. Using the same size categories as Read *et al*. (1987) the biplot of axes 1 and 2 was marked to show the position of each size range; the result is shown in Figure 7.20. Size group 1 (the largest) is well represented on the

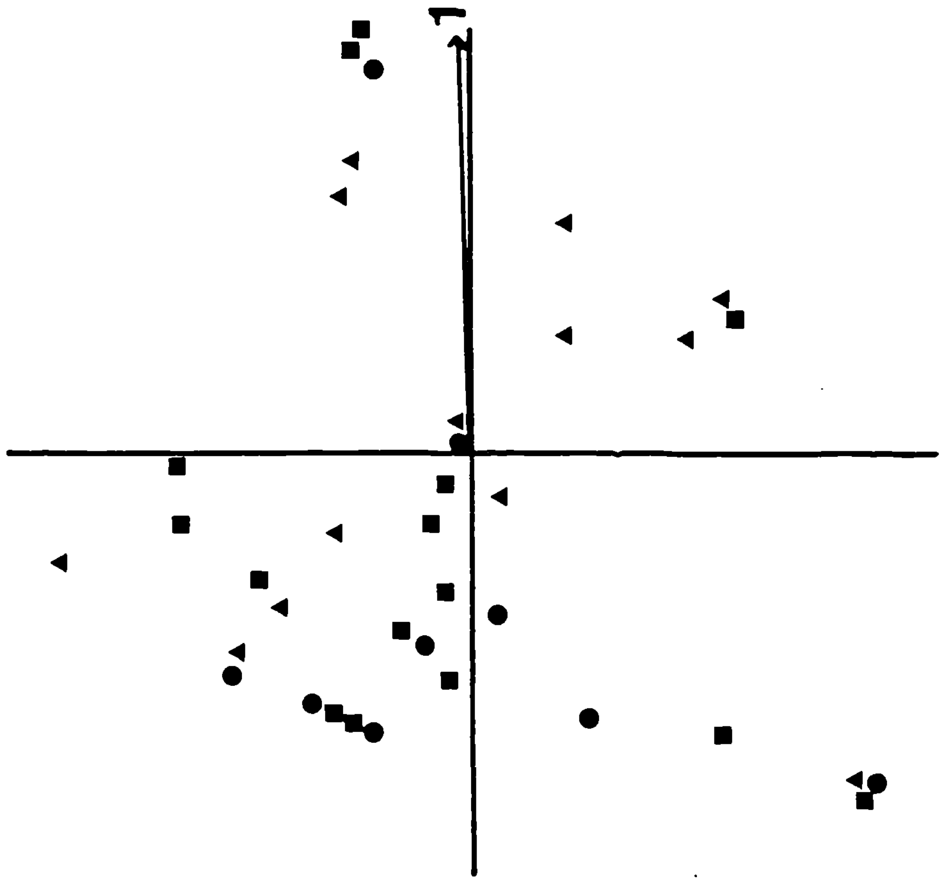
Figure 7.20 CCA of carabids (as in Figure 7.19a) showing size groupings.

■ Size I >12.5mm
● Size II 7.5-12.5mm
▲ Size III 6.0-7.4mm
□ Size IV <6.0mm

Figure 7.21 CCA of carabids (as in Figure 7.19a) showing breeding seasons.

■ Spring
● Summer
▲ Autumn


1



high pollution side of the biplot but large species do occur elsewhere. Correlations of each body size group with the principal component axis 1 were not significant ($r>0.05$, $p>0.05$). Two of the most widespread species (*Pterostichus madidus* and *Abax parallelepipedus*) are in size group 1 and are found centrally on the plots. The other size groups appear scattered. Another point of interest arising from Read *et al*. (1987) is the time of breeding in Carabids. This can be considered as spring, summer or autumn according to the species (most carabid beetles have a one year life cycle). Beetles were assigned to their appropriate breeding season (given in Read *et al*. 1987) and these were marked on the biplot (Figure 7.21). At first glance there appears to be a low number of summer breeders associated with high levels of pollution, but *Cychrus caraboides* and *A. parallelepipedus* are both summer breeders and were abundant at Haw wood. Correlations of numbers of each breeding category were not significantly correlated with principal component axis 1 ($r<0.71$, $p>0.05$). The negative correlation between numbers of spring breeding individuals and metal concentrations shown in Read *et al*. (1987) does not show up in the species data on the biplots.

One point which is easily overlooked when studying the biplots is that the species which are abundant at the polluted sites but also abundant elsewhere tend to maintain a central position. For example, relating to the carabid biplot, *A. parallelepipedus* was captured at Haw wood and it was the most frequently caught species. Because it was also caught in large *numbers at other sites it is positioned* centrally in the biplots, the relative importance of species such as this one in polluted environments must not be underestimated.

7.5e ix. Spiders. (Figures 7.22a and b).

For the spiders as well as most of the other CCA runs, the first environmental variable has a large influence on the first ordination axis. In contrast to many of the other plots, a large number of species appear to be in the same region as site 6 (HW), and this site is close to site 5 (PW). The overall pattern of the species when plotted on ordination axes 1 and 2 is triangular. One point of the triangle extends towards sites 5 and 6, one point towards site 1 (WM) and the third in a direction away from all three environmental variables. Many species can be identified as associating with high levels of pollution, but several of these were recorded in very low numbers (ie. 1 or 2) at site 6 only. Of those caught in larger numbers Coelotes atropos, Anyphoena accentuata, Micragus herbigradus, Lepthyphantes zimmermanni, and Robertus lividus are positively correlated ($r>0.812$, $p<0.05$) with environmental variable 1 and several of the metal concentrations separately. A few other species (e.g. Agroeca brunnea, Centromerus sylvaticus and Stemonyphantes lineatus) are represented in this portion of the plot but do not show significant positive correlations.

Figure 7.22 CCA of spiders.

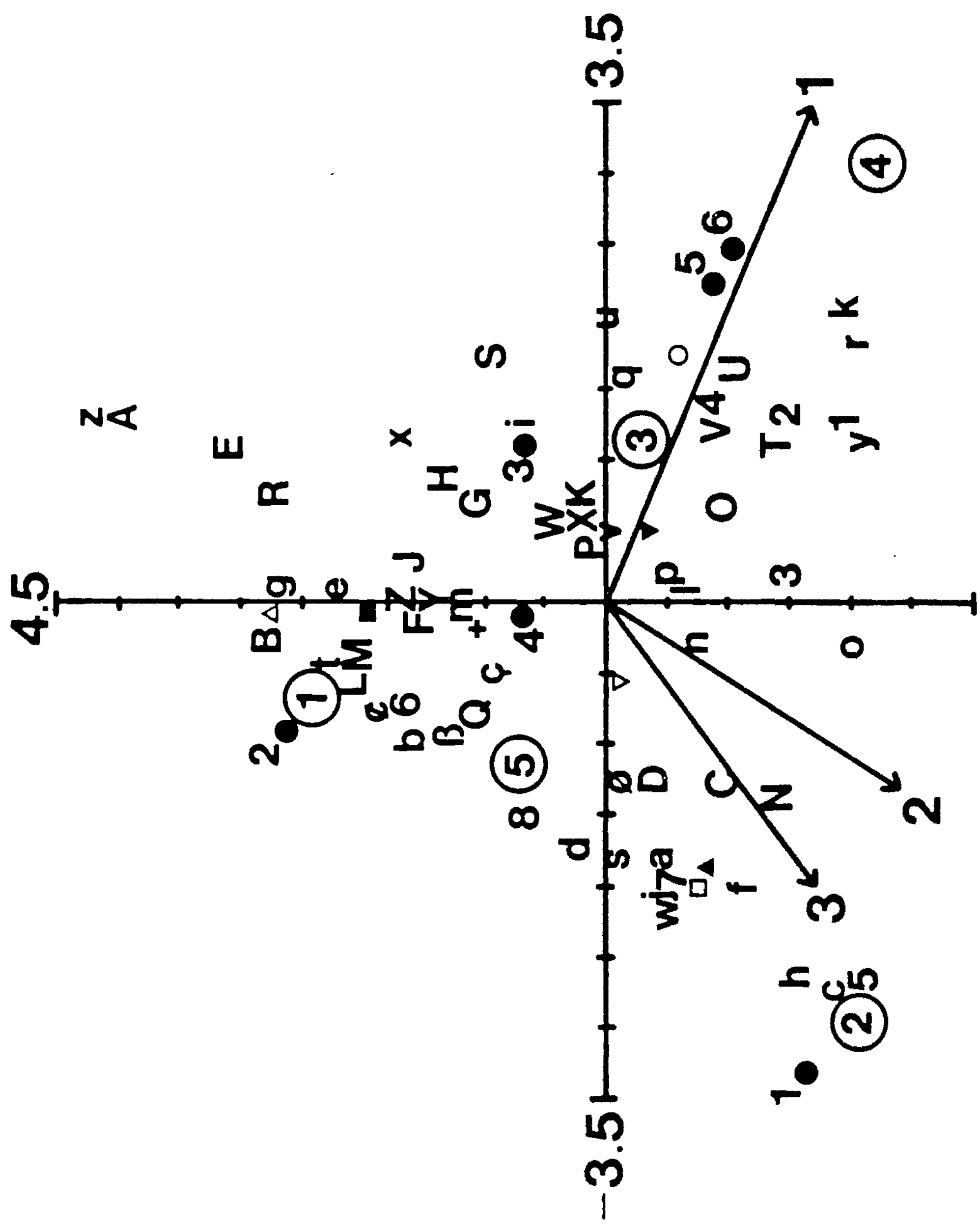
7.22a Axis 1 horizontal, axis 2 vertical. Species marked on using symbols indicated below.
7.22b Axis 1 horizontal, axis 3 vertical. Separate species not indicated due to the closeness of many of the points.
Sites as in Figure 7.7

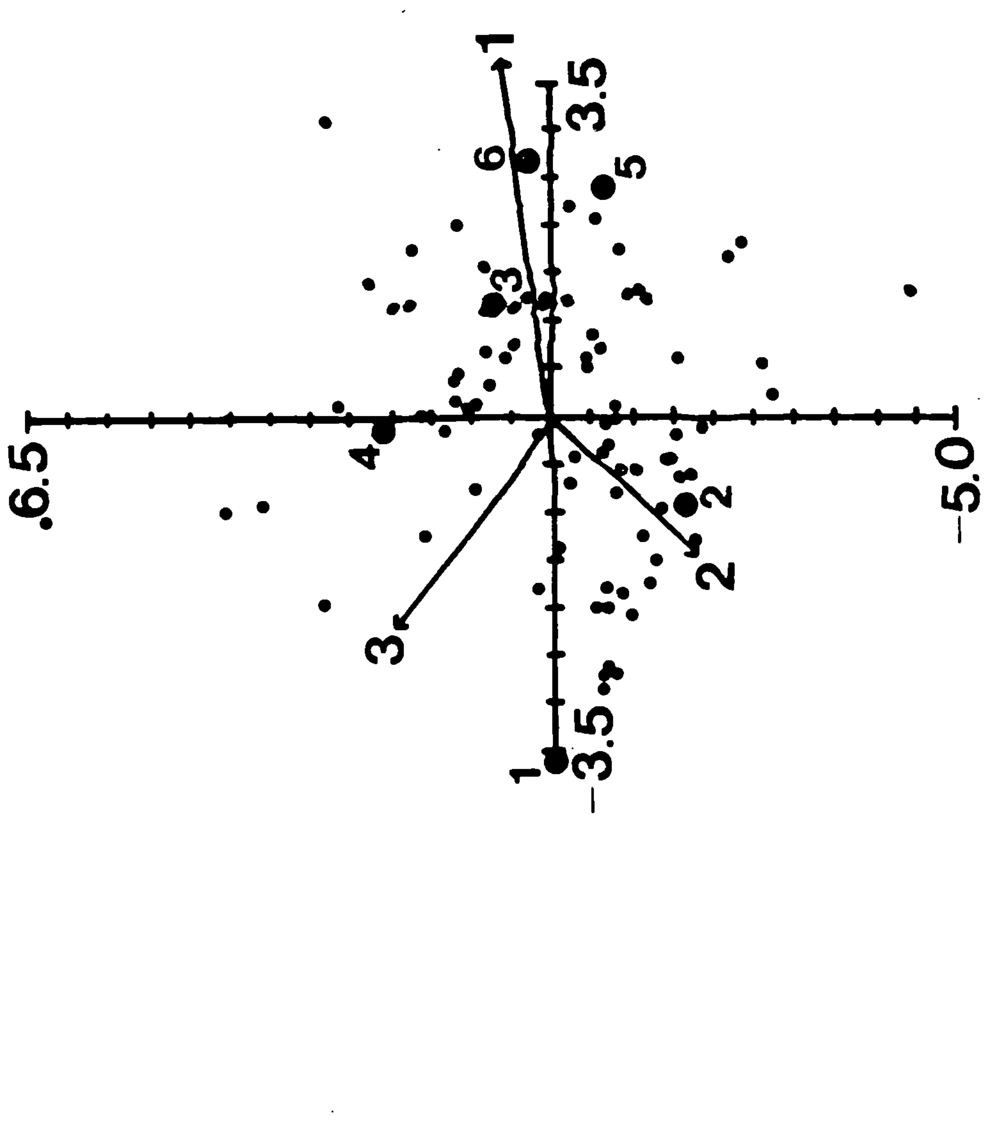
Environmental variables:
1 PCA axis 1
2 PCA axis 2
3 PCA axis 3

Species:
a *Pardosa nigriceps*
b *P. amentata*
c *P. lugubris*
d *P. pullata*
e *Trochosa ruricola*
f *T. terricola*
g *Alopecosa pulverulenta*
h *Pirata hygrophilus*
i *Tegenaria silvestris*
j *T. atrica*
k *Coelotes atropos*
l *C. terrestris*
m *Robertus neglectus*
n *Enoplognatha ovata*
o *Theridion pallens*
p *Clubiona compta*
q *C. terrestris*
r *Agroeca brunnea*
s *Zora spinimana*
t *Pisaura mirabilis*
u *Ero furcata*
v *Meta segmentata*
w *M. mengei*
x *Pachygnatha degeeri*
y *Anyphaena accentuata*
z *Segestria senoculata*
A *Walckenaera unicornis*
B *Pocadicnemis pumila*
C *Diplocephalus latifrons*
D *D. picinus*
E *Dicymbium nigrum*
F *Monocephalus fuscipes*
G *Walckenaera dysderoides*
H *Maso sundervalli*
J *Oedothorax fuscus*
K *Oedothorax retusus*
L *Gongylidium rufipes*
M *Ceratinella brevipes*
N *Macragus rufus*
O *Gonatium rubellum*
P *Walckenaera acuminata*
Q *Savignya frontata*
R *Erigone atra*
S *E. dentipalpis*
T *Walckenaera nudipalpis*
U *Centromerus sylvaticus*
V *C. dilutus*
W *Linyphia clathrata*
X *L. hortensis*
Y *Lepthyphantes flavipes*
Z *L. tenuis*
■ *L. mengei*
▲ *L. tenebricola*
▼ *L. pallidus*
△ *L. ericaeus*
▽ *L. cristatus*
□ *L. alacris*
○ *L. minutus*
\+ *Bathyphantes gracilis*
₵ *B. parvulus*
Ç *B. nigrinus*
Ø *B. concolor*
β *B. approximatus*
1 *Stemonyphantes lineatus*
2 *Labulla thoracica*
3 *Oreonetides abnormis*
4 *Helophora insignis*
5 *Agyneta subtilis*
6 *Meioneta saxatilis*
7 *Porrhoma convexum*
8 *Linyphia triangularis*

Lists of species which are too close to distinguish between:
1. *Pardosa prativaga*
Hygrolycosa rubrofasciata
Linyphia montana
Ceratinella scabrosus
Meioneta rurestris
Porrhomma pallidium

	Lepthyphantes obscurus	*Drapetisca socialis*
	Ceratinella brevis	*Dismodicus bifrons*
2.	*Pardosa hortensis*	*Pachygnatha listeri*
	Pirata latitans	*Walckenaera cuspidata*
	Xysticus lanio	*Erigonella hiemalis*
	Cyclosa conica	*Floronia bucculenta*
	Araneus diadematus	*Trochosa spinipalpis*
3.	*Robertus lividus*	*Lepthyphantes zimmermanni*
	Micraqus herbigradus	*Microneta viaria*
4.	*Pholcomma gibbum*	*Amaurobius fenestralis*
	Zelotes pusillus	*Diplocephalus cristatus*
	Philodromus aureolus	
5.	*Episinus angulatus*	*Pachygnatha clercki*
	Clubiona lutescens	*Wiehlia calcarifera*
	C. pallidula	*Linyphia peltata*


4.5
−3.5
3.5
−3.0



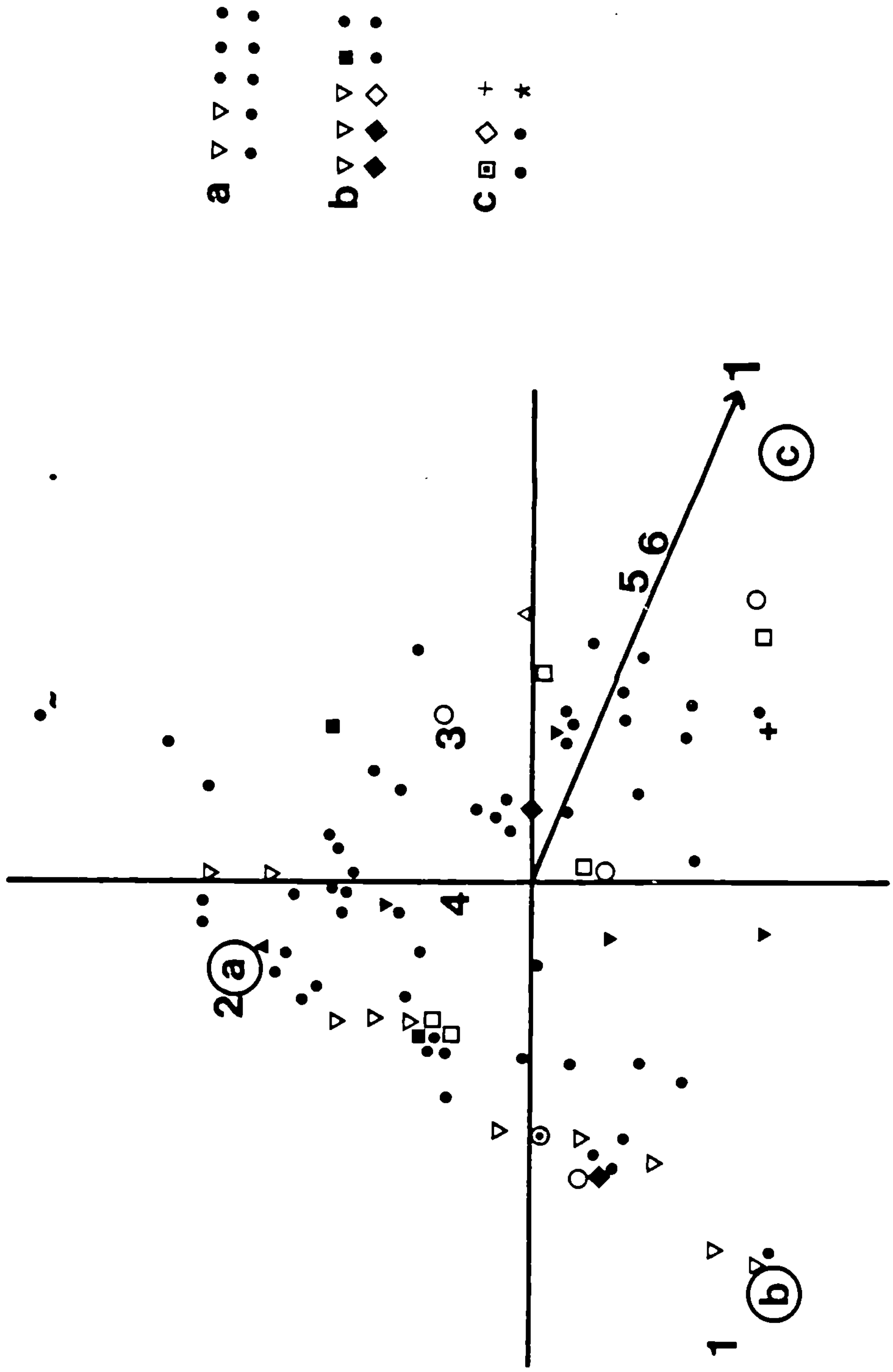
6.5
-5.0
3.5
-3.5
1
2
3
4
5
6

At the other side of the biplot there is a large number of species all appearing to respond almost equally against the pollution effect, but more widely separated by ordination axis 2. The result of this is a well defined border to the upper left side of the triangle in Figure 7.22a. The same effect is not seen in Figure 7.22b because of the difference in orientation and the fact that the third ordination axis does not separate the species so well. A larger number of species ´dislike´ the high metal concentrations, these are shown in figure 7.22a. Many genera are represented but one notable point is the number of species belonging to the genus Pardosa. Amongst the lycosid or wolf spiders, many individuals were captured belonging to the genus Pardosa, often in large numbers. Although activity is usually restricted to a small proportion of the year during the spring and early summer, this period was equally well sampled in the present study at all sites. The triangular shape implies that the spider diversity is higher at the clean sites. As reported in section 7.4 spider H´ was negatively correlated with zinc concentrations ($r>0.833$, $p<0.05$).

Figure 7.23 shows the same biplot as Figure 7.22a marked according to the family of each spider species. The preponderance of Linyphiids is obvious, and species in this family occur in most areas of the biplot. Of great interest is the distribution of lycosids on the plot. All species in this family are found away from the pollution effect and the

Figure 7.23 CCA of the spiders (as in Figure 7.22a) showing the family to which each species belongs.

● Linyphiidae	- Dysderidae
▽ Lycosidae	◉ Zoridae
○ Agelenidae	✚ Anyphoenidae
▲ Pisauridae	◇ Thomisidae
▼ Theriidae	△ Mimetidae
⊡ Gnaphosidae	□ Clubionidae
■ Tetragnathidae	* Amaurobidae
◆ Argiopidae	+ Saltic idae


a
b
c
1
2
3
4
5
6
a
b
c
1

majority are on the well-defined border noted before. Pisaura mirabilis, the only representative of the family Pisauridae in the present study is in a similar position. This family is very closely related to the family Lycosidae. Although a total of 16 families of spiders were represented in all the collections, for most of them only a few species were caught and the positions of these are scattered across the plots. The number of spiders (individuals and species) in each family caught at the sites is shown in Table 7.11. A 5x6 chi-squared test was calculated (using individuals) for those families which supported 5 or more individuals at most of the sites. The result was significant (X^2 = 2320, df = 20, $p<0.001$) showing that the difference between the observed and expected numbers of individuals in each family is significant, ie. the sites appear to have a different distribution of individuals in the families. The various proportions of animals in each family are illustrated in Figure 7.24.

The lycosids form a large proportion of the ground running spiders in woodlands (Uetz 1977). The linyphiids mostly use webs to catch their prey, whereas the lycosids use sight, although they often sit still and wait for the prey to come close by (Edgar 1969). The almost complete absence of this family from the fauna in polluted sites (5 and 2 individuals caught at sites 6 and 5 respectively compared to 515 and 662 from sites 1 and 2) would seem to indicate a large difference in the spider populations and there might seem to be a vacant niche if these animals are absent. This may be filled by other spiders or other taxa. A few other ground running spiders were captured during the study, for example those in the family Clubionidae and the atypical tetragnathids of the genus Pachygnatha. Of particular note though are the large agelinids in the genus Coelotes, two species of which were found in the present study although only one was present in reasonable numbers. The distribution of Coelotes atropos over the sites is almost a mirror image of that of the lycosids, with 149 individuals captured from each of sites 5 and 6 but none from sites 1 and 2. Figure 7.25b shows the numbers of Coelotes atropos and lycosids caught at each site.

C. atropos is a large bodied species up to 13mm in length, belonging to the family Agelinidae. It has smaller eyes than the lycosids and they are less widely spaced. It usually lives under stones and logs and builds a tubular web. The spider sits in the web burrow which has a thicker collar at the entrance and there is no typical Agelinid sheet web outside (Roberts 1985b). In C. terrestris, a closely related British species, the immature spiderlings live in the same web as the mother for a while and are fed by her with regurgitated food (Burgess 1978).

C. atropos was the largest spider regularly caught at the most polluted site (HW, site 6) and it is possible that it is partially assuming the role of the lycosids. Although

Table 7.11

NUMBERS OF ANIMALS AND SPECIES IN EACH SPIDER FAMILY

Number of species shown in brackets.

FAMILY	WM	KW	KG	TP	PW	HW
Dictynidae			1(1)		2(1)	6(1)
Dysderidae		2(1)				
Gnaphosidae						1(1)
Clubionidae*	9(2)	4(2)	8(2)	8(4)	9(3)	20(3)
Anyphaenidae	1(1)				1(1)	2(1)
Thomisidae	10(1)					1(1)
Salticidae	1(1)					
Lycosidae*	515(11)	662(10)	53(9)	49(6)	2(2)	5(4)
Pisauridae	1(1)	8(1)	3(1)	2(1)		
Agelenidae*	2(1)	4(2)	1(1)	13(2)	150(2)	152(2)
Mimetidae		1(1)	1(1)			3(1)
Zoridae	3(1)	2(1)		1(1)		
Therididae*	18(3)	17(3)	24(2)	13(4)	34(2)	41(4)
Tetragnathidae	195(1)		2(1)	6(2)		1(1)
Argiopidae	6(4)	6(2)	3(1)	4(1)	8(1)	4(1)
Linyphiidae*	856(36)	742(45)	896(40)	887(35)	1022(29)	1006(34)

X^2 for families shown as * = 2320.89, df = 20, $p<0.001$

Figure 7.24 Bar chart to illustrate the distribution of the spider families throughout the sites.

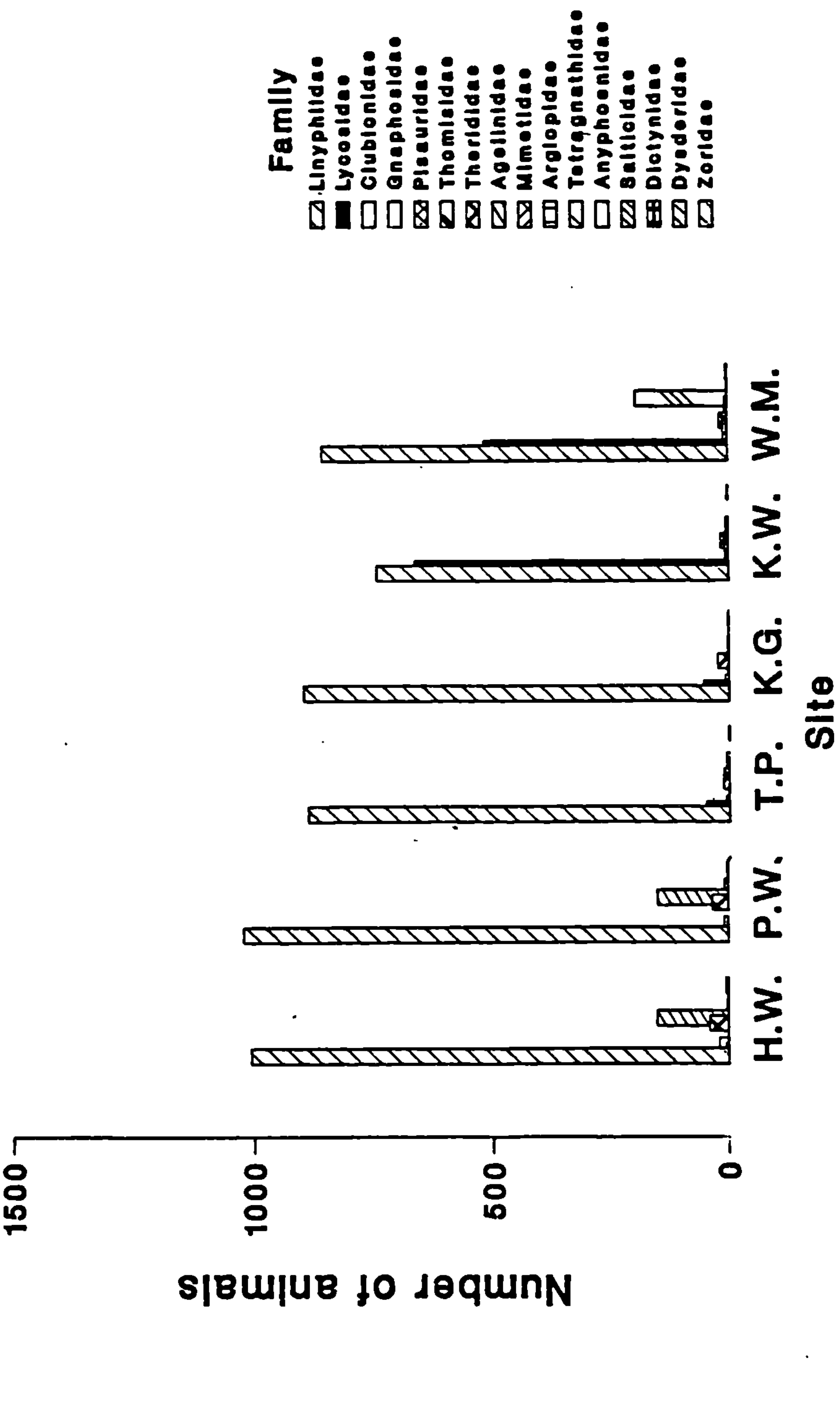
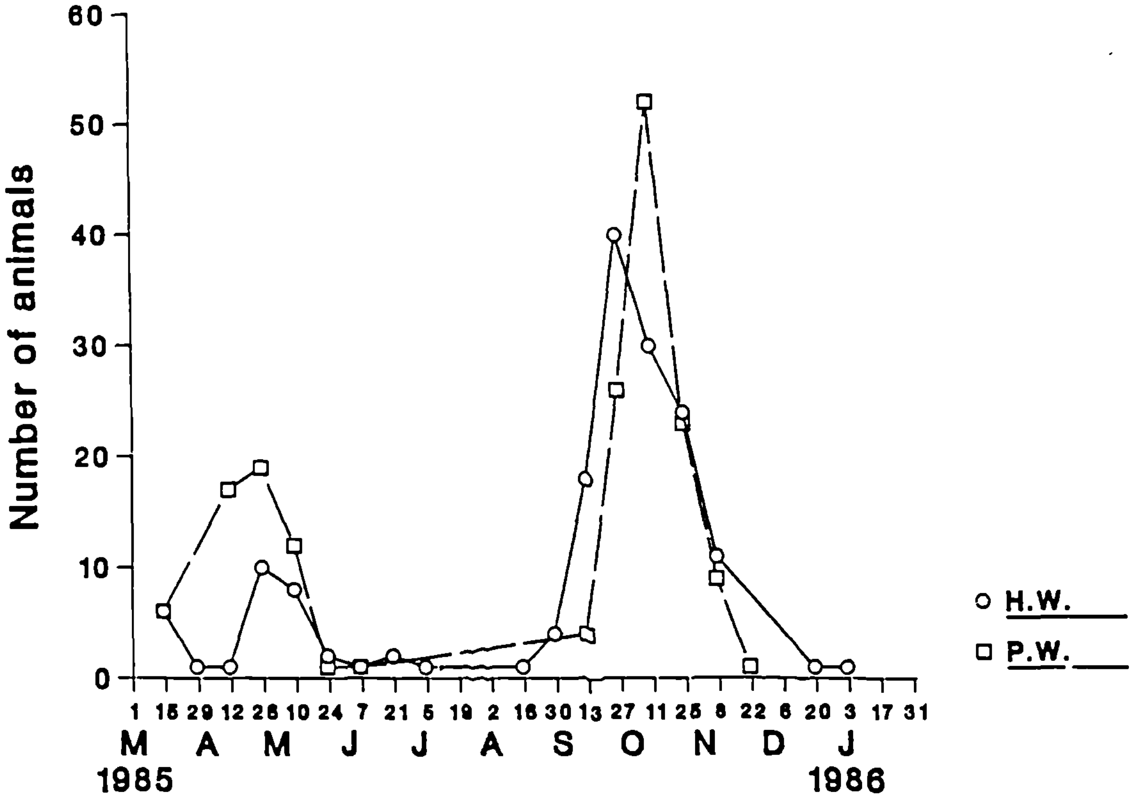
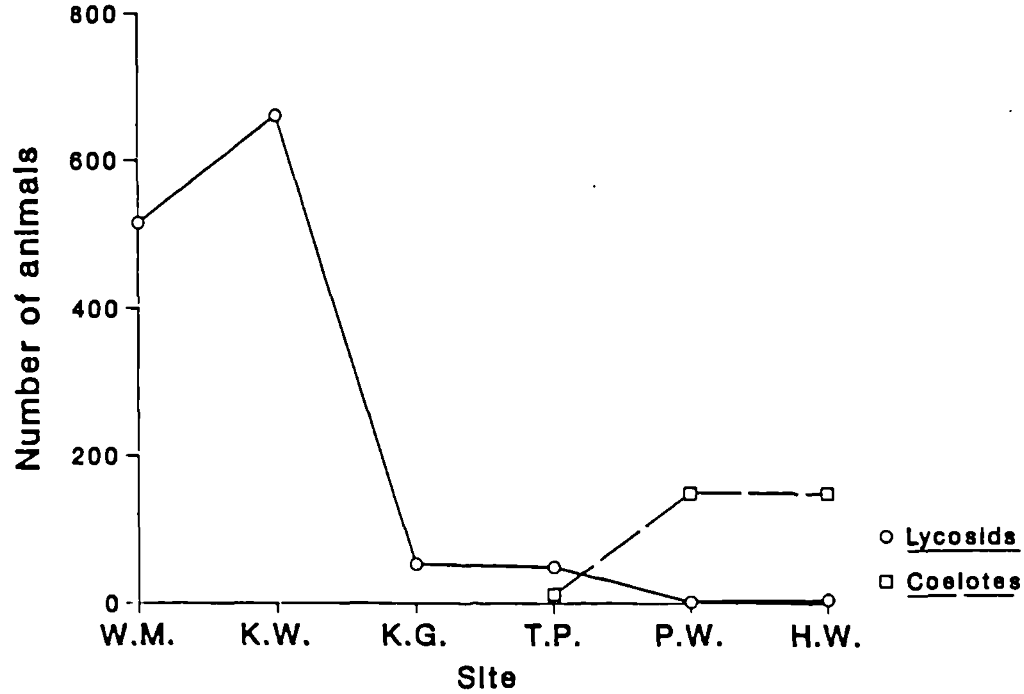

Number of animals
0
500
1000
1500
H.W.
P.W.
T.P.
K.G.
K.W.
W.M.
Site
Family
Linyphiidae
Lycosidae
Clubionidae
Gnaphosidae
Pisauridae
Thomisidae
Theridiidae
Agelinidae
Mimetidae
Argiopidae
Tetragnathidae
Anyphaenidae
Salticidae
Dictynidae
Dysderidae
Zoridae

Figure 7.25a Graph to show the numbers of *Coelotes atropos* caught at each site at different times of the year.

Figure 7.25b Graph to show the numbers of Lycosidae and *Coelotes atropos* caught at each site.


Number of animals
60
50
40
30
20
10
0
1 15 29 12 26 10 24 7 21 5 19 2 16 30 13 27 11 25 8 22 6 20 3 17 31
M A M J J A S O N D J
1985
1986
H.W.
P.W.


Number of animals
800
600
400
200
0
W.M. K.W. K.G. T.P. P.W. H.W.
Site
Lycosids
Coelotes

the species has a retreat, it may forage away from it; animals kept in the laboratory were seen to emerge from the burrow and capture prey. The increase in number of clubionids and the presence of a gnaphosid species may also be related to the lack of lycosids.

It is interesting that Jones (1983) records _C. atropos_ as being active in spring and early summer, with eggs laid in early summer. In both sites where the species was recorded in large numbers (PW and HW) more animals were active in the Autumn. This is illustrated in Figure 7.25a. The lycosids captured at the unpolluted sites were active in the spring and early summer as expected. One of the findings of Read _et al_. (1987) was that some species of carabid beetles were active later in the year at polluted sites and that _Nebria brevicollis_ may not enter diapause at Haw wood. It appears that a similar phenomenon may be occurring with the spiders. _C. atropos_ is perhaps more adaptable than the lycosids and has been able to alter its habits so that it may live in areas where the lycosids cannot. This species (_C. atropos_) has also been found on mine sites in the Mendips, and therefore its representation in polluted woods in the present study may not be entirely due to chance.

Hunter _et al_. (1987c) caught lycosids in pitfall traps in a grassland area close to a copper refinery and recorded that peak activity occurred in May (this may be based on combined data from polluted and control sites however). The animals were not identified to species and it is possible that different lycosid genera or species were present there. Fairly low numbers of lycosids were caught by Bengtsson & Rundgren (1984) in the area close to a brass mill in Sweden, but low numbers were also caught in control sites. More information about the life cycle and activity of this species would be an asset.

7.5f The position of the sites in the CCA plots discussed above.

In most of the CCA plots for different groups of animals the first axis (ie. the metal pollution factor) has had the largest influence on the first ordination axis. Despite this the sites are not always ordered in the manner of the first principal component analysis, that is with site 6 (HW) well separated from the others. While site 6 is usually well apart, site 1 (WM) is also on several occasions separated, for example in the woodlice, the harvestmen and also the centipedes and carabids. In several plots sites 5 and 6 (PW and HW) are close together (e.g. the woodlice, harvestmen and to a lesser extent the millipedes).

These results may illustrate that while site 6 differs from the others principally in the amount of heavy metal pollution, site 1 (WM) is also rather different from many of the other woods. The metal pollution at Haw undoubtedly does have an influence on the animals there but all of these sites have species not found at other sites. Site 1 (WM) probably has more than most since it may sometimes stand apart, but site 6 also has a large number which were not found elsewhere. It would seem that four of the sites studied were very similar, but that two were more different from each other and from the other four. It is still an unresolved problem as to how many of these differences are caused by the heavy metal pollution.

7.5g Data all pooled. (*Figures 7.26a and b*).

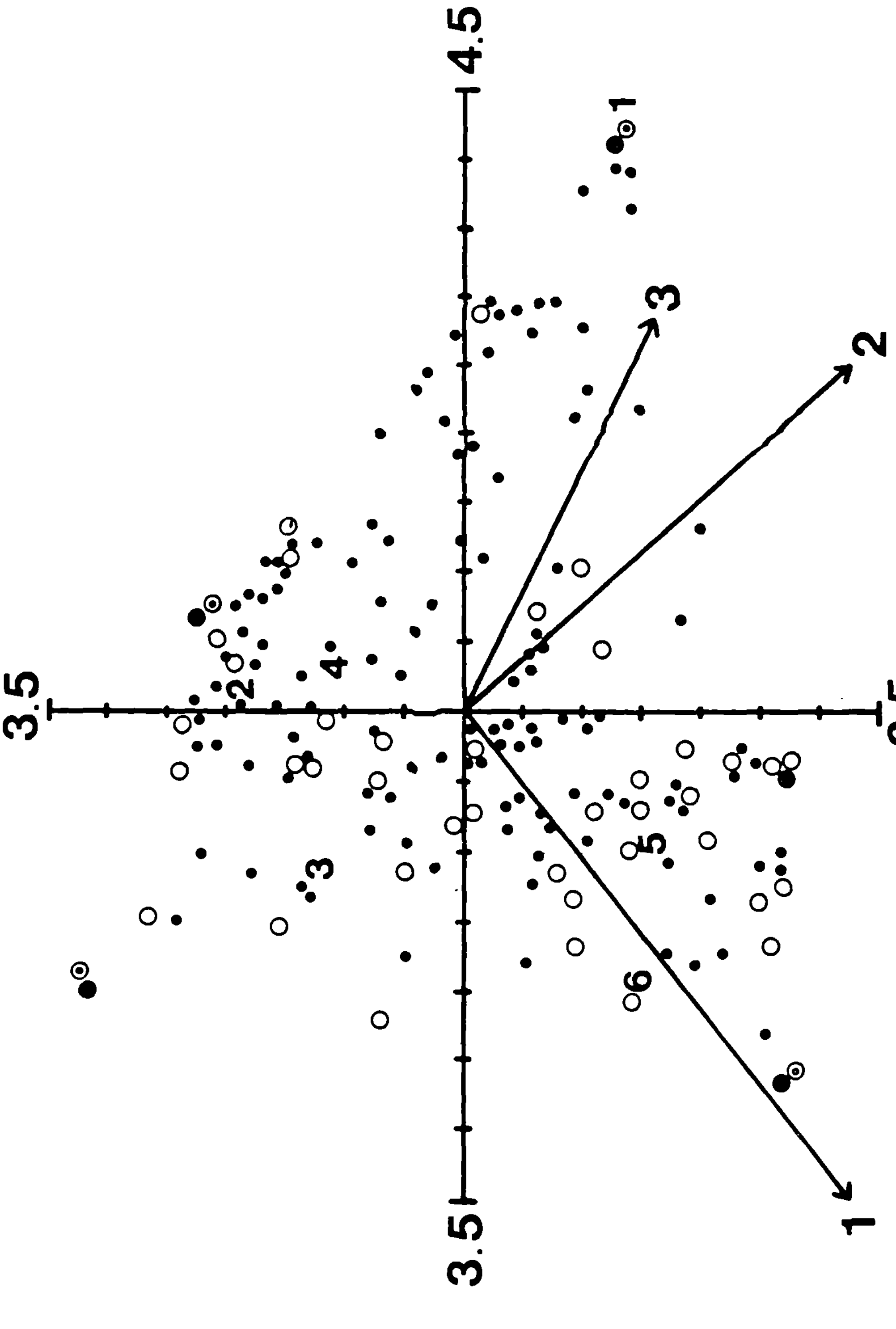
The biplots produced for the CCA using all the species pooled (ie. all the data used in the previous runs) show a similar pattern to those produced using the spider data. The plot of axes 1 and 2 has a roughly triangular shape, with one vertex extending towards site 6 (HW), and opposite it a well defined linear boundary. The pooled run of CCA differs from the runs using individual groups, since all the species are used in calculating the ordination axes and the position of the environmental variables. Species may therefore occupy a different position from that on biplots produced from smaller groups of species. The plots of pooled data encompass a wide range of organisms with very different roles in the community. The triangular shape here probably arises largely from the high abundance of spider species, and again clearly demonstrates a marked fall in species diversity with increasing pollution.

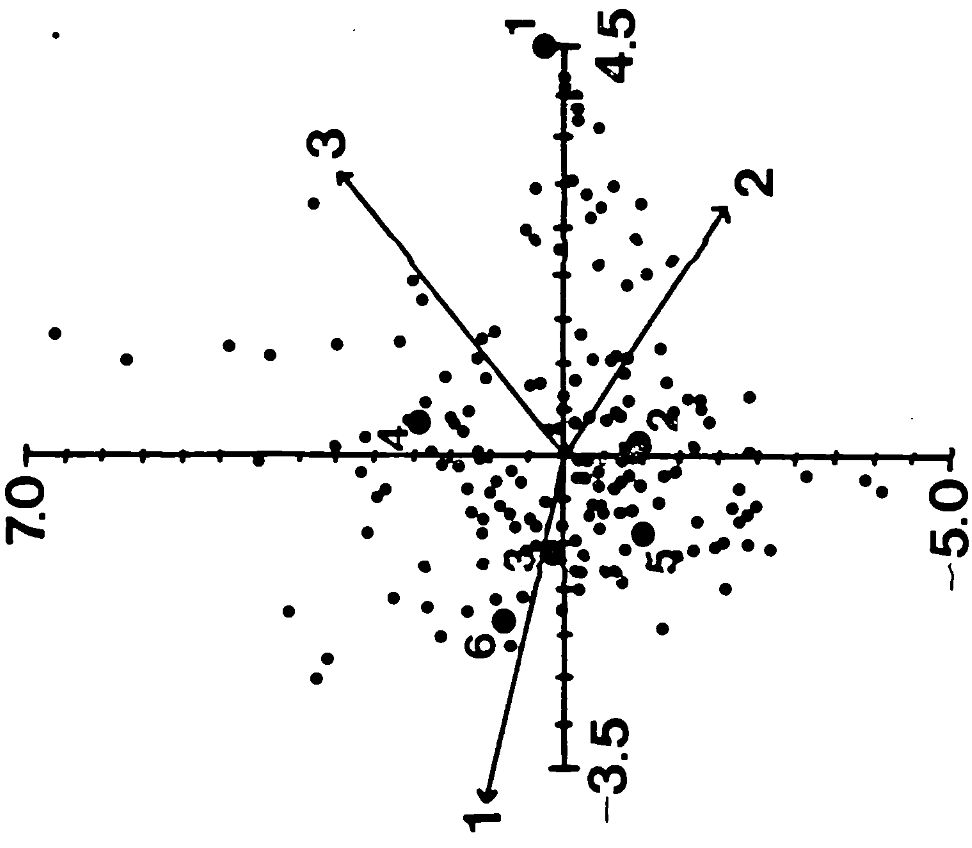
Figure 7.26 CCA of all the species pooled.

7.26a Axis 1 horizontal, axis 2 vertical. Species are not named because of the difficulty markinging each point. Decomposers and predators are marked separately as indicated below. Where several species occur very close together a separate symbol was used.
7.26b Axis 1 horizontal, axis 3 vertical. Species are not marked separately, predators and decomposers are not destinguished.

• Predators ○ Decomposers

● Several predators ◎ Several decomposers


4.5
3.5
3.5
3.5
1
2
3


7.0
−5.0
4.5
−3.5
1
2
3
4
5
6

One of the initial reasons for the commencement of the present study was to investigate the decomposer community. It is of interest to identify those organisms classified as decomposers on the biplot and compare their distribution with that of the predators. This has been done in Figure 7.26a. There is a wide scattering of both groups throughout the plot, and so it would seem likely that the polluted sites are not deficient in terms of decomposer species. In fact it appears that there are more decomposer species in this region of the plot than close to site 4 (PW). It is tempting to conclude that decomposers are less affected by the pollution than the predators, many of which are on the extremes of the plot.

The shape of the biplot of axes 1 and 2 conveys considerable information on faunal composition. Site 1 (WM) appears to be rather distinct in this respect from the other sites. This is perhaps to be expected, since Wetmoor wood is a well documented ancient woodland with many indicator species amongst the plants (Hendry 1980). Apart from coppicing it has been far less disturbed than any of the other woods sampled and it is a large area of woodland which is itself totally enclosed by other areas of woodland. It would be interesting to discover if the fauna of another ancient oak-hazel wood is similar to that of Wetmoor; it is probable that it would appear at a similar position on the CCA biplot.

Site 6 (HW), the most heavily polluted site, forms another point of the triangle in Figure 7.26a, and is close to site 5 (PW). These two sites are both polluted in comparison to the rest of the woods studied, but they are less similar with regard to vegetation or soil type. The invertebrate fauna is similar in some respects, though the element of difference is indicated by the wide separation of the two sites by ordination axis 3 (principally influenced by environmental axis 3, measuring tree density, soil type etc.). In this last respect site 6 (HW) is more similar to site 3 (KG) although these are more widely separated by the metal concentration governed axes.

Environmental variable 1 (that relating to metal concentrations) has the effect of producing a long ´tail´ in its direction of influence. This ´tail´ consists of species which are associated with heavily polluted sites; the species at the extreme end consist mainly of those which are recorded only from site 6 (HW) and nowhere else. Closer in to the origin are species which were found at site 5 (PW) as well. On the opposite side of the origin to the point of environmental variable 1 are the species which ´dislike´ pollution and these have formed a sharp boundary to the edge of the triangular shape. These species occupy a range of sites (1-4), and reflect considerable diversity in these unpolluted sites.

It might be postulated that because species are under selection pressure to become better adapted and more tolerant of the pollution at the heavily polluted sites, the ´tail´ extending parallel to environmental variable 1 will be patchy as some species have managed to become more tolerant than others of the increased pollution. On the opposite side, where species are sensitive to pollution, there are no selection pressures to become more sensitive. A species cannot become more sensitive than it already is when there is no pollution.

In highly polluted areas, the effect of the pollution is a strong influencing factor on the community. Species there have been able to withstand the encroachment of pollution or are chance colonisers which are able to survive in its influence. Interspecific competition may be expected to be at a low level and the community consists of pollution tolerant forms of those which are preadapted in some way to the situation. In areas of low pollution, unless there is another environmental restriction (e.g. very low pH) the number of potential species is much greater. The factors which determine which species are found are not dependent on degree of pollution (presumably below some threshold level) but on either different environmental variables or increased pressure of interspecific competition. Thus the shape of the biplots illustrates the breadth of potential species that may be found in deciduous woodlands in the Avon area: a smaller number are able to thrive in polluted woods, but for some reason are out-competed in unpolluted sites. Close to the origin of the plots are those species with widespread distributions which have been able to thrive in both polluted and unpolluted areas, and include, for example *Abax parallelepipedus*, *Oniscus asellus* and *Lithobius variegatus*.

7.5h General discussion and conclusions.

CCA clarifies the relationships between species and attendant environmental variables. Coupled with bivariate correlations this method is a powerful tool for ecologists interested in why certain species are found in particular places. It was unfortunate for this application that the method adopted requires more sampling sites than environmental variables. But by simplifying the environmental measures by calculating PCA and using the resulting three dominant axes as input variables in the CCA this could be overcome. Indeed, the use of just three variables was advantageous because the biplots were a projection of a simple 3-dimensional space, and the environmental variables were uncorrelated: this helped considerably in the interpretation. Sampling of additional sites would have proved difficult because few similar sites were available and there would have been an inevitable reduction in data collected from each one. The collection of animals over a large portion of the year is important to ensure that differences in activity times are not reported as differences in species numbers.

CCA computation for the smaller species groups (for example millipedes and woodlice) are revealing in terms of relationships between sites, species and environmental variables. The small number of points on each diagram do not indicate low numbers caught, on the contary, several thousands of animals of some species were caught, but rather that these groups are represented in Britain by a few species.

In contrast, the spiders and carabids have many more species in Britain, a point reflected by the density of points in the relevant biplots. The CCA of these groups have, on the whole, been more useful because it is possible to examine subgroups (for example delineated by size or family).

Pooling all the invertebrates identified and calculating CCA gives an impression of the communities as a whole. Although much information is shown in the resulting biplots it is important not to regard the results in isolation. Other measures of the community, for example absolute numbers and diversities, can still help in identifying characteristics of the communities.

7.5i Summary.

1. The numbers of arthropod species caught at each site appeared to decline with increasing proximity to the

smelter, but this was not statistically significant. The number of individuals was very variable between the sites.

2. Shannon's diversity index H' *was calculated for each* group of animals and t-tests between them were computed. There was a tendency for the diversity of most groups to decrease at sites closer to the smelter. The woodlice, harvestmen and spiders gave significant negative correlations between diversity and some metal concentrations.

3. Ordination of the sites was attempted on the basis of environmental factors, using principal component analysis (PCA). A large proportion of the variation was explained by the first axis which incorporated most of the effects of the metal concentrations.

4. The values of the site scores on the first 3 PCA axes were used as environmental variables to compute canonical correspondance analysis (CCA) using various groups of animals.

5. For each group, species could be identified which were associated with or disassociated from high levels of pollution. Bivariate correlations corroborate the CCA results.

6. Species´ positions on the biplots may be related to aspects of their ecology, and indicate why species respond to pollution in the way that they do.

7. For smaller groups (e.g. millipedes and woodlice) the information gathered was related to levels of pollution, but those groups with larger numbers of species proved most informative.

9. An attempt was made to relate body size and time of breeding in carabids to the plots and to metal pollution. This did not reveal any additional information.

10. In several CCA biplots there were fewer species represented on the side of the plot representing high pollution.

11. For several groups, different species belonging to the same genus responded very differently to some of the environmental variables, including those relating to pollution.

12. Division of the spider data into families showed different distribution of the various families, in particular the Lycosidae. Lycosids are almost completely absent at polluted sites, where they appear to be partially replaced by <u>Coelotes atropos</u> an agelenid.

13. A summary of the position of the sites in the CCA carried out reveals that site 1 (WM) is rather different from most of the others. Site 6 (HW) is usually also apart from the others owing to the high metal concentrations there.

14. By pooling the invertebrates into one data set, identified to species level, a triangular plot of axes 1 and 2 similar to that of the spiders alone is produced. One vertex includes site 1 (WM) and another site 6 (HW). Environmental variable 1, corresponding to the major metal effects, extends into the vertex containing site 6 and opposite to it is a sharply defined ´side´ of the triangle.

15. The shape of the distribution of species is interpreted as indicating that a few species can survive in areas of high pollution whereas a far larger number occur where there is no pollution. Other factors must affect the distribution in the latter case; these may include other environmental variables or biotic ones such as competition.

16. CCA is a powerful tool in the studies of community ecology but should not be used in isolation. It is of greatest use when many species are involved in the analysis.

Chapter 8.

OVERVIEW AND CONCLUSIONS.

8.1. The effect of heavy metal pollution on the decomposers.

Decomposer communities are very diverse and complex. Despite the enormity of the subject, this study has tried to shed a little more light upon the reaction of a particular group of organisms to the pressure of pollution. Concentration on invertebrate decomposers has enabled several taxocenes to be studied in detail by a variety of trapping methods. By consistently sampling in the same 6 woodland sites a picture can be compiled of each one and the position of the more polluted ones assessed.

Sampling of the microarthropods, the mites and collembola, has illustrated one of the problems involved in the comparison of decomposer communities between two sites. This is the problem of litter depth. Because an increase in pollution levels has a tendency to result in deep litter layers, the most polluted site (HW) has over four times the depth of the site furthest from the smelter (WM). Those animals at the polluted site have more leaf litter to inhabit and thus a greater potential food source. Although differences in the number of microarthropods at the sites were found, both per unit area and per unit volume of litter, these differences were not easily related to metal concentrations.

The worms, sampled by formalin extraction, also showed variation with degree of metal pollution; these too could not be attributed directly to the metal pollution.

A much longer term survey was undertaken for the macroarthropods. Pitfall sampling over a full year provided abundant material. The main decomposers sampled in this way were isopods, diplopods and molluscs. The latter, whilst not sampled in the most efficient way showed some interesting results and are probably worth a more concentrated study in the future. The macroarthropod community did not appear to be substantially reduced by the pollution. In many aspects, the polluted site seemed to have many species which were able to thrive. The fauna at this site was unusual in many respects, supporting several unlikely species, an aspect which will be returned to shortly.

Metal concentrations within the selected decomposers (one woodlouse species, one millipede species and the worms) were examined and revealed differences in the response to the four metals measured. Cadmium in all instances was accumulated, whereas zinc, copper and lead were less consistent. Laboratory experiments indicated that some millipede species may have a low juvenile survival rate at the polluted site.

8.2 The effect of heavy metal pollution on the predators.

Invertebrate predators were also sampled by pitfall trapping. The main groups represented at the sites were spiders, harvestmen, beetles and centipedes. As with the decomposers, no obvious lack of organisms was revealed in the more polluted sites, although in several cases more species appeared to be related to the cleaner sites. By the use of canonical correspondance analysis (CCA) some of the sites could be seen to have identifiable communities or species groupings. The most polluted site (HW) was separated from the others by higher metal concentrations but less obviously by other environmental factors. Inadvertent sampling of small mammals at the sites showed higher metal concentrations in the predacious species at the polluted site which was considered to be related to the food consumed.

Although one of the primary tasks of the project was to determine the effect of pollution on the decomposers, it is considered by many workers that the predators may be more affected. Thus metals are often considered to accumulate along food chains affecting the top carnivores the most. This has recently been disputed by Van Straalen & Van Wensem (1986) who considered the method of feeding was very important. The presence of fewer predator species close to the polluted sites on the pooled CCA biplot might indicate that, for some reason, predators are less able to adapt to increased pollution than decomposers. It is interesting that both shrew species of the genus Sorex were present at Haw wood. S. araneus in particular was seen to accumulate high concentrations of some metals but was obviously able to reproduce, even if at a reduced rate. Some species appear able to survive in highly polluted areas despite expectations to the contary (cf. Dysdera crocata and Lithobius variegatus *Hopkin & Martin 1984b, 1985*).

8.3 Biological indicators.

Many studies of pollution have tried to establish biological indicators. Usually this has involved measuring concentrations of heavy metals within a certain organism and relating this to the degree of pollution in the environment. The use of lichen species has developed as a means of indicating approximate levels of atmospheric sulphur dioxide pollution in an area and attempts are also being made to use aquatic species in a similar way. In the present study two types of ´indicators´ have been highlighted as an aside to the main theme. Both these types appear potentially useful but require further confirmation of this potential in other regions, they are preliminary ´pointers´ of interest.

The first group are those suggested by the log normal plots (Table 7.2). In this instance the species represented are those which increase in numbers at low levels of pollution, thus the use of them may be limited to certain circumstances. Further examination of these species may prove interesting.

The second type of indicators are those extracted from the CCA biplots. These are potentially more useful because the mere presence of a specific species may indicate certain levels of pollution. It is well known that ancient woodlands may have particular species associated with them. Wetmoor possesses a few such species, e.g. Xysticus lanio, despite this, some more common species were also identifiable as ´disliking´ pollution. With the inclusion of further polluted sites, it may be possible to determine many species, the presence of which can indicate the degree of pollution. This might be less useful for woodland communities but an extension to more frequently encountered habitats e.g. waste ground might provide interesting results. This method may be less accurate in determining precise level of contamination than measuring the metal concentration of a particular species, and/or its habitat however, once established less equipment is necessary and the cost is reduced.

8.4 What are the factors which determine which species are found at the polluted site (Haw wood)?

It was evident from the outset of the project that some of the animals found at Haw were uncommon. Whilst other sites yielded a few surprises, for example one of the few British records of *Wiehlea calcarifera* a 1mm long linyphiid spider from Tockington Park, such species may be expected when pitfall trapping for extended periods. Most of the species from Haw wood were not rare, but did not occur at any of the other sites sampled (or were in reduced numbers at other sites). Perhaps the most obvious example was *Chordeuma proximum* a millipede with a predominantly south westerly distribution in Britain. Individuals were caught so regularly at Haw that nearly all the stadia were represented and the life history could be established. Such studies add to the knowledge of millipede biology and can also help elucidate pollution problems.

In a habitat which is polluted to a level in excess of that tolerated by many organisms, there are several main possibilities to explain the presence of a species. The animals can either have existed in the area prior to the pollution and due to wide degree of pollution resistance, or a developing tolerance, been able to withstand the increasing levels. Alternatively it could have arrived after the pollution but be pre-adapted in some way to cope with the polluted environment.

Whatever the situation it would appear that the more adaptable a species was, the more likely it would be able to respond to the new situation. In habitats such as Haw wood, the expected species would be those with high turnover rates, short life cycles, probably producing large numbers of offspring. This would enable the fast evolution of new strains adapted to the situation. Because of this, when a particular species is able to thrive well, it has the capacity to increase quickly thus becoming dominant in the community. As the levels of pollution are maintaining the main competitors, in the form of other species, at a lower population level, the species which is dominating can remain in position for a longer time. Thus relatively resistant species that are non-competitive may thrive. This phenomenon is the cause of the increased dominance of certain species seen in polluted environments. In a situation where the level of pollution has declined relative to the past, as may have occurred at Haw, any ´new´ species are likely to be good colonisers. Thus we can predict the types of animals we would expect to find at Haw, so we are now in a position to examine those that were caught to see if they fit this prediction.

Table 8.1 shows those species that were captured in large numbers at Haw (above c. 100 individuals) together with

Table 8.1

DETAILS OF LIFE HISTORY PARAMETERS FOR THOSE SPECIES COMMON AT HAW WOOD

	AGE TO MATURITY (years)	LENGTH OF LIFE (yrs)	NO. OF EGGS	BREEDING SEASON	REFERENCE	NOS. CAUGHT
Millipedes						
Glomeris marginata	2-3	10-11	Max 86	SP/SU	1	482
Chordeuma proximum	1	1	Max 54	June	2	313
Polydesmus angustus	2	3	200	SP/SU	3	216
Woodlice						
Oniscus asellus	1-2?	4	$\bar{x}$ 45	SU	4	1008
Trichoniscus pusillus	1-2	1-2	4-11	SP/SU	5	216
Centipede						
Lithobius variegatus	2	5-6	?	SP/SU/AU	6	93
Carabids						
Nebria brevicollis	1	1	?	AU	7	153
Abax paralellipipedus	1-2	1-2	?	SP/SU	8	228
Harvestmen						
Nemastoma bimaculatum	1	1-2	?	Jul or Oct	9	733
Lacinius ephipiatus	1	1	?	AU	10	146
Rilaena trianglaris	1	1	?	AU	10	190
Spiders						
Coelotes atropos	1?	1?	?	SU	11	149
Microneta viaria	?	?	?	?		161
Lepthyphantes zimmermanni	?	?	?	W?		610

SP = Spring. SU = Summer. AU = Autumn. W = Winter.

Sources of information: 1. Heath et al. 1974, 2. Read in prep., 3. Blower 1985, 4. Beyer 1957, 5. Sutton 1968, 6 Eason 1964, 7. Wheater 1984, 8. Wheater 1987, 9. Williams 1962, 10. Todd 1949, 11. Jones 1983.

those details of the life history that can be gleaned from the literature. The first point to notice is the paucity and uncertainty that exists of the details even of a very common species such as the linyphiid spiders, despite a search of the available literature. Several of the species involved probably have relatively short life cycles. This is true of most beetles, spiders and harvestmen. *Nemastoma bimaculatum* is unusual in living rather longer than most species of harvestmen. Amongst the millipedes and woodlice, the species present do not necessarily represent those well recognised for their abilities as colonisers which is surprising. *Polydesmus angustus* has a shorter life cycle than many species and has a widespread distribution. Both the woodlice species are also widespread and Sutton (1968) has shown that *Trichoniscus pusillus* can be adaptable in its life history according to different conditions.

All the species listed in Table 8.1 are very common in southern Britain with the exception of *Chordeuma proximum* and *Coelotes atropos* which are rather more restricted in distribution. It is possible that these two species have ´benefited´ particularly from the effects of the pollution.

8.5 Final discussion.

One of the biggest problems in a field study on the effect of pollution on a living system is proving that the differences that have been found are due to the pollution being measured. There are so many factors which determine the distribution of animals and the composition of the community that it is impossible to measure them all. Many indeed are unknown. By the use of several different methods, both in the laboratory and the field, the knowledge of the invertebrate community in Avon woodlands polluted by heavy metals has been extended. The computation of CCA together with correlations has determined as far as possible that pollution has had an effect on these communities.

The composition of the community at Haw wood is undoubtedly different from that of the surrounding woods. The problem is that every wood is unique in its invertebrate fauna. Haw wood has a disproportionately high number of some species and a sprinkling of rare ones which do not occur in the surrounding woods.

The level of heavy metal contamination at the most polluted site (HW) is considerably lower than that experienced in many other studies, for example in U.S.A. or Sweden. Because of this a large scale decline in numbers of animals has not been recorded. Existence of a mixed woodland closer to the smelting works might have altered the situation. Despite this, evidence of the effect of the pollution has been noted at site 5 (PW) several miles away from the pollution source. It is important not to underestimate the effect lower levels of pollution is having on invertebrate communities which are more subtle than large scale decline in numbers.

One important point to stress from the work is the importance identification to the species level. It is slowly being realised that closely related species may behave very differently when confronted with the same environmental conditions. Lumping animals together into trophic levels or even major taxonomic groups, as so many workers have done in the past, can disguise a multitude of reactions and effects.

This thesis has inevitably created more questions than it has answered. Paramount amongst these is our lack of fundamental biological information on many of the common invertebrates. In spite of this, I hope it has added a little to our knowledge both of the invertebrates communities and of the animals themselves

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APPENDIX 1.

TRANSFORMATION OF DATA.

Data consisting of metal concentrations in animals or plants traditionally includes a proportion of outlying points, causing the population as a whole to deviate from a normal distribution. This being so, it is not valid to undertake certain statistical analyses which require data to be normally distributed. Two alternative approaches have been used in the present study to overcome this problem.

First nonparametric techniques can be employed because they do not make the assumption that the data are normally distributed. For example, the Spearman rank correlation coefficient was used several times in preference to the standard Pearson coefficient and Mann-Whitney U was calculated as an alternative to the t-test. Mann-Whitney U tests for the difference between two populations based on their medians rather than their means as the t-test does.

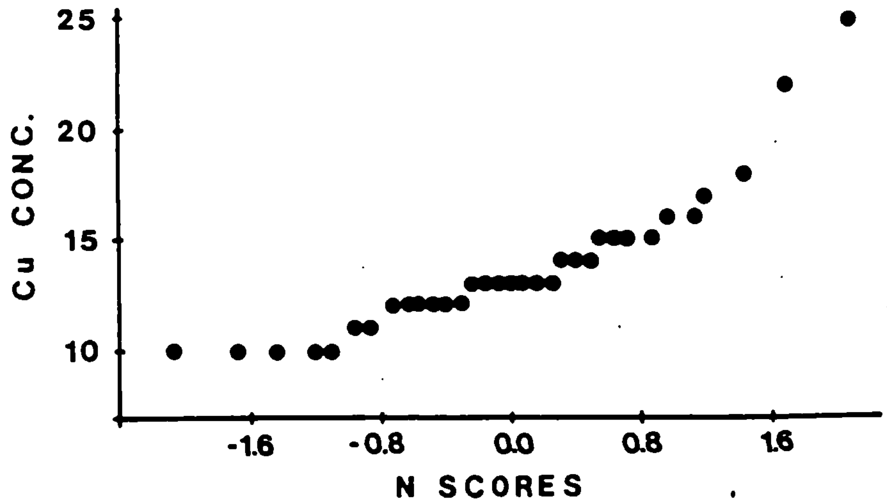
A second method of overcoming the problem is to *transform* the data in some manner, in order to make it more normally distributed. One way to do this, which was employed in this study is to use logarithms to the base 10 of the individual data points. This enables, for example, analysis of variance to be carried out on the data set.

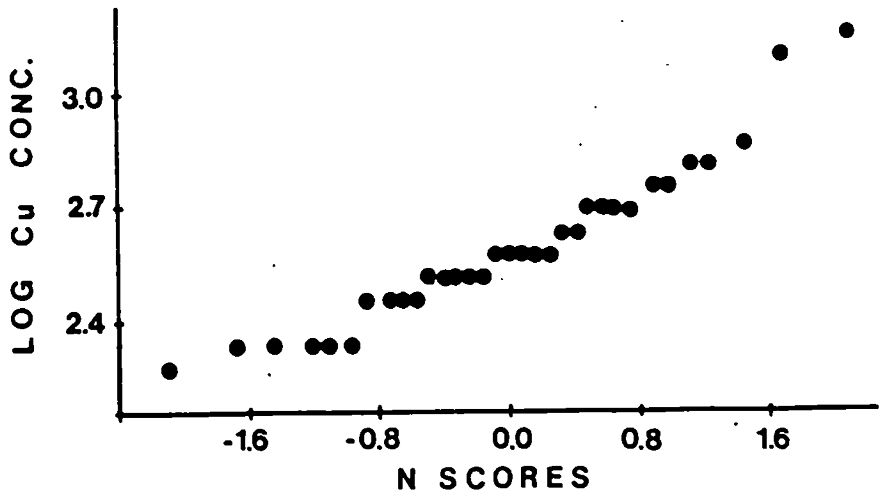
Besides simply plotting a histogram of a data set there is another method of examining it for normality, which is to plot the data against their normal scores. With small and/or scattered sets of data this method is easier to use. The normal scores are those that would be obtained, on average, if the sample was from a normal distribution. An example is shown in Figure A1.1, where the copper concentrations in the 'rest of body' component of Sorex araneus from Kington Grove are plotted against their normal scores (calculated using minitab). For a normally distributed sample a straight line is produced. Considerable curvature can be seen in the plot in Figure A1.1. When the data are transformed by logging and the new normal scores plotted, the result is Figure A1.2. Clearly the plot is now straighter. A disadvantage of this method is that the plots still have to be examined subjectively for normality.

Plots of data against their normal scores.

Figure A1.1 Untransformed data.

Figure A1.2 Log transformed data.


25
20
15
10
Cu CONC.
-1.6
-0.8
0.0
0.8
1.6
N SCORES


3.0
2.7
2.4
LOG Cu CONC.
-1.6
-0.8
0.0
0.8
1.6
N SCORES

APPENDIX 2.

TESTING THE PITFALL TRAP CAPTURES FOR OVER TRAPPING.

2.1 Introduction and methods.

The original intention of the sampling strategy used in this thesis was that the pitfall traps would be left in position for over a year. By obtaining collections at the beginning and end of the trapping period which corresponded to the same times of the year comparisons could be made in order to check that the traps were not denuding the area of animals. Unfortunately, due to the vagaries of the British weather combined with additional human factors in the form of trap and site vandalism, only one fortnightly trapping period at the end coincided with one at the beginning. The animals caught in the first two week period ending 3.1.85 were totalled as were those caught in the two week prior to 2.1.86. Due to the low numbers of animals active at this time of the year, those caught were grouped into predators (spiders, carabids and centipedes), decomposers (millipedes, woodlice and molluscs) and harvestmen.

2.2 Results and discussion.

A paired t-test was calculated for each 'group' of animals, comparing those animals caught in the 1985 collection with those caught in 1986. The results are shown in Table A2.1a. All three t-tests were significant, indicating that more animals were caught in one collection than the other. For

Table A2.1

A. RESULTS OF PAIRED t-TESTS TO TEST FOR OVERTRAPPING

SITE	PREDATORS 1985	PREDATORS 1986	DECOMPOSERS 1985	DECOMPOSERS 1986	HARVESTMEN 1985	HARVESTMEN 1986
WM	28	13	17	8	62	14
KW	30	18	13	23	54	18
KG	38	19	15	17	102	19
TP	47	32	16	16	29	19
PW	63	49	15	21	21	12
HW	51	27	18	27	41	32
Paired t-Test						
t	9.35		2.67		2.68	
p	0.001***		0.05 *		0.05 *	

B. RESULTS OF CHI-SQUARED TESTS TO EXAMINE OVERTRAPPING AT EACH SITE

Site	X^2	p
WM	8.66	0.05 *
KW	15.44	0.001 ***
KG	20.4	0.001 ***
TP	1.03	0.05 ns
PW	3.64	0.05 ns
HW	7.47	0.05 *

the predators and the harvestmen, more individuals were trapped in 1985 at every site. However more decomposers were caught in 1986. The t-tests help to show differences in absolute numbers of animals, but by calculation of chi-squared tests for each site, differences in the relative numbers of each group can be detected. The results of chi-squared tests are shown in Table A2.1b. The results calculated for Tockington Park and Pegwell wood were not significant but the other tests were.

It appears that the harvestmen and the other grouped predators may have been over trapped during the year, however the decomposers have not and seem to have increased in numbers. This has caused little variation in the relative proportions of predators and decomposers at two of the sites (TP. and PW.) but has altered the composition at the other sites.

Whilst these results indicate that some degree of over trapping may have taken place, the changes in numbers and composition of the animals may not be due solely to this. Differences in weather conditions and microclimate within the woods may account for some of the differences in activity and abundance. A much more influential factor is probably that the collection taken in January 1985 was the first taken. Although the traps had been in place for a few days prior to the two week trapping period, this collection is still within the 'digging in' period thus the catches are not representative of the normal catches at the sites. It is unfortunate that the only comparable trapping times were so early in the year and included the first trapping collection. The standard trapping period does not include either of these two collections and covers the time of the year when most animals are emerging. The calculations shown above do not conclusively demonstrate that over trapping occurred. Because identical situations were used in each site, the degree of overtrapping, if it exists must be assumed to be the same at each site therefore the method of capture still permits comparisons between the sites.

APPENDIX 3.

Table A3.1

MONTHLY WEATHER DATA FROM LONG ASHTON, ALTIDUDE 20M

(GRID REFERENCE ST 544 703)

Month /Year	Mean Monthly Max.Temp (°C)	Mean Monthly Min.Temp (°C)	Total Rainfall (mm)	Total Sunshine(hrs)
Dec. 1984	9.3	2.2	86.7	44.2
Jan. 1985	4.3	-1.8	69.5	52.0
Feb. 1985	6.6	1.6	51.5	83.8
March 1985	9.2	1.4	62.6	119.4
April 1985	13.0	4.9	46.2	151.2
May 1985	15.7	6.9	64.8	185.7
June 1985	17.3	8.9	130.3	182.6
July 1985	20.9	12.1	77.9	217.9
August 1985	18.4	11.7	132.0	177.6
Sept. 1985	19.3	10.7	31.5	142.6
Oct. 1985	14.2	8.1	53.9	79.2
Nov. 1985	8.1	2.2	46.7	81.3
Dec. 1985	9.7	5.0	163.9	31.8
Jan 1986	7.1	1.4	121.9	42.6

Table A3.2

MONTHLY WEATHER DATA FROM CHELTENHAM, ALTITUDE 65M

(GRID REFERENCE ST 746 218)

Month /Year	Mean Monthly Max.Temp (°C)	Mean Monthly Min.Temp (°C)	Total Rain-fall (mm)	Total Sun-shine(hrs)
Dec. 1984	8.6	2.7	50.4	66.4
Jan. 1985	3.7	-2.1	33.0	49.2
Feb. 1985	5.7	-0.4	41.9	91.1
March 1985	8.9	1.4	73.1	110.3
April 1985	12.8	4.9	59.6	142.0
May 1985	15.6	7.1	90.2	201.7
June 1985	17.4	8.6	145.1	163.6
July 1985	21.4	12.0	58.1	220.6
August 1985	18.9	11.5	107.9	117.1
Sept. 1985	19.9	10.2	24.9	88.8*
Oct. 1985	15.1	7.3	49.5	67.0
Nov. 1985	7.5	1.6	47.5	78.5
Dec. 1985	9.4	4.7	109.2	29.4
Jan 1986	6.7	1.1	97.4	54.0

*Records for 17 days only.

APPENDIX 4.

Table A4.1

VEGETATION RECORDED IN 30 X 30 M AT EACH SITE

Numbers of trees listed, shrubs (½ canopy or less) in brackets, ground vegetation as presence or absence only.

	HAW WOOD	PEGWELL WOOD	TOCKING-TON PARK	KINGTON GROVE	KNAPP WOOD	WETMOOR WOOD
Quercus robur	7	1	7	1		18
Quercus petraea					)	
Quercus robur x petraea					) 18	
Quercus cerris		1		1	)	
Fraxinus excelsior	9 (30+)	7 (25)	8	4	6	3
Betula pendula						1
Carpinus betula		16 (2)				
Sorbus torminalis						1
Acer pseudoplatanus	1 (6)	(8)		4 (26)		
Acer campestre	1	(7)	1		(5)	2
Crataegus monogyna	(3)	(10)	(3)	(4)	(30)	(1)
Corylus avellana	(24)	(1)	(22)	(7)		(76)
Ilex aquifolium		(1)	+			+
Ligustrum vulgare	+	(2)				
Ulmus glabra			(1)	+	(10)	
Sambucus nigra	(3)	(31)	(6)	(8)	(8)	
Viburnum lantana	(1)					
Rubusfruticosus sens. lat.	+	+	+	+	+	+
Carex pendula			+			+
Geum urbanum		+	+		+	+

Table A4.1 contd

	HAW WOOD	PEGWELL WOOD	TOCKING-TON PARK	KINGTON GROVE	KNAPP WOOD	WETMOOR WOOD
Betula pubescens						+
Chamaenerion angustifolium		+	+	+		+
Potentilla sterilis						+
Primula vulgaris	+					+
Galium aparine		+	+	+	+	
Rumex pulcher			+		+	
Arrhenatherum elatius				+	+	
Meracleum sphondylium	+	+			+	
Urtica dioica	+	+	+	+	+	
Tamus communis	+		+	+	+	
Rosa sp.		+		+	+	
Festuca gigantea		+	+		+	
Mercurialis perennis	+	+	+		+	
Hedara helix	+	+	+	+	+	
Glechoma hederacea			+	+	+	
Milium effusum	+	+	+	+	+	
Ranunculus repens	+	+	+		+	
Cirsium arvense					+	
Bryonia cretica	+				+	
Geranium robertianum		+		+	+	
Arctium minus			+		+	
Stachys sylvatica	+	+	+		+	
Brachypodium sylvaticum		+	+		+	
Poa nemoralis	+	+	+	+	+	
Prunus spinosa					+	

Table A4.1 contd

	HAW WOOD	PEGWELL WOOD	TOCKING-TON PARK	KINGTON GROVE	KNAPP WOOD	WETMOOR WOOD
Agrostis stolonifera						+
Carex sylvatica	+		+		+	+
Ajuga reptans						+
Narcissus pseudonarcissus						+
Hypericum hirsutum					+	+
Athyrium filix-femina						+
Salix cinerea						+
Lotus uliginosus						+
Lysimachia nemorum						+
Viburnum opulus						+
Taraxacum sp.		+	+			+
Luzula pilosa						+
Veronica officinalis						+
Epilobium hirsutum						+
Bromus ramosus					+	+
Galium pallustre						+
Dryopteris dilatata	+	+	+		+	+
Circaea lutetiana	+	+	+		+	+
Viola riviniana/reichenbachiana						+
Deschampsia caespitosa			+			+
Hypericum pulchrum						+
Juncus effusus						+
Anemone nemorosa						+
Lonicera periclymenum	+		+	+	+	+

Table A4.1 contd

	HAW WOOD	PEGWELL WOOD	TOCKING-TON PARK	KINGTON GROVE	KNAPP WOOD	WETMOOR WOOD
Lapsana communis					+	
Anthriscus sylvestris					+	
Arum maculatum	+	+			+	
Holcus mollis				+		
Silene dioica	+	+	+	+		
Hyacinthiodes non-scripta	+	+	+	+		
Stellaria media				+		
Solanum dulcamara				+		
Pteridium aquilinum				+		
Dryopteris filix-mas	+	+	+	+		
Ribes rubrum				+		
Oxalis acetosella				+		
Salix caprea	+		+	+		
Lamiastrum galeobdolon	+			+		
Polygonum persicaria			+			
Ribes nigrum	+					
Fagus sylvatica		+				
Castanea sativa		+				
Veronica montana		+				
Ranunculus auricomus		+				
Alliaria petiolata		+				
Cornus sanguinea	+					
Clematis vitalba	+					
Euonymus europaeus	+					
Daphne laureola	+					

Table A4.2

PLANT SPECIES FOUND IN $1m^2$ QUADRATS AT EACH SITE

Results for each of 3 quadrats given separately values as percentage cover.

SPECIES	HAW WOOD			PEGWELL WOOD			TOCKINGTON PARK			KINGTON GROVE			KNAPP WOOD			WETMOOR WOOD		
Rubus fruticosus	80	65	60	20	12	39	82	45	55	67	70	70	70	88	88	65	40	55
Hedera helix		5	20	12	12	20		1		3	10	30						
Hyacinthoides non-scripta	2	2	1				3	4	5	5	7	2						
Fraximus excelsior	1		1	1	10	1		1	1			1	1			10	10	10
Arum maculatum	1												1					
Mercurialis perennis		1		1									5		5			
Lamiastrum galeobdolon			5					4	15									
Geranium robertianum				65	46	15												
Galium aparine				2	15	15	8		1		1		7	10	1			
Brachypodium sylvaticum				1		7												
Poa nemoralis					5													
Milium effusum						1	1	5	7		1							
Crataegus monogyna						3												
Silene dioica							2		3					1				
Urtica dioica							1						10	2	3			
Lonicera periclymenum							2								1		7	1
Glechoma hederacea									10									
Pteridium aqualinum										25								
Acer pseudoplanatus										1	1							
Holcus mollis											2							
Rumex pulcher													7					
Ranunculus repens													1					

Table A4.2 contd

SPECIES	HAW WOOD	PEGWELL WOOD	TOCKINGTON PARK	KINGTON GROVE	KNAPP WOOD	WETMOOR WOOD
Juncus effusus						1 1
Carex pendula						3 25
Galium palustre						1 1 1
Luzula pilosa						1 1
Chamaenerion angustifolium						3
Hypericum pulchrum						12
Viola sp.						8
Anemone nemorosa						1
Brachythecium rutabulum	3					
Plagiomnium undulatum			1			
Eurhynchium praelongum			2 12 80			1 35
Atrichum undalatum						30 1
Thuidium tamariscinum						3 10

APPENDIX 5.

Table A5.1

METAL CONCENTRATIONS IN THE MOSS BAGS FROM EACH SITE

Given as means of the five bags at each site for each collection minus the mean of all the controls.

Sites 1 = Wetmoor Wood 2 = Knapp Wood 3 = Kington Grove

4 = Tockington Park 5 = Pegwell Wood 6 = Haw Wood

Dates are date of collection. Moss Bags were in place for the four weeks prior to this date.

COLLECT. DATES	SITE	CADMIUM	COPPER	LEAD	ZINC
28.03.85	1	0	7.22	0	8.6
	2	0.02	6.17	0	0
	3	0.52	6.26	0	31.0
	4	0	6.58	0	0
	5	0.43	3.21	0	0
	6	2.42	22.83	74.8	151.6
25.04.85	1	0.11	4.2	0	0
	2	0	2.2	60.7	0
	3	0.52	1.2	16.7	59.9
	4	0.57	3.06	64.0	22.1
	5	0.69	5.03	22.8	24.7
	6	5.37	34.05	213.3	399.2
23.05.85	1	0.22	0	55.5	38.3
	2	0	0	87.1	10.0
	3	0	0	0	0
	4	0.14	0	0	0
	5	0.47	0	205.5	17.1
	6	1.72	9.27	105.1	122.0
20.06.85	1	0.69	8.93	343.2	67.2
	2	0.23	3.25	235.9	74.6
	3	0.99	5.85	663.7	96.3
	4	0.37	6.85	442.9	37.0
	5	1.96	7.67	842.4	154.6
	6	2.11	16.94	599.2	166.9
18.07.85	1	1.11	0	182.5	9.6
	2	0.5	0	274.3	129.5
	3	0.2	0.82	323.5	60.6
	4	0.31	0.11	157.7	119.2
	5	0.46	0	174.7	139.9
	6	2.19	7.68	80.8	218.2

Table A5.1 contd

COLLECT. DATES	SITE	CADMIUM	COPPER	LEAD	ZINC
15.08.85	1	0.12	5.31	0	0
	2	0	4.05	0	0
	3	0	1.37	0	0
	4	0	1.62	0	0
	5	0.47	2.26	0	16.2
	6	2.62	17.06	104.3	305.3
12.09.85	1	0	0	0	25.4
	2	0.2	0	6.4	26.2
	3	0.21	0	2.5	63.1
	4	0	0	0	0
	5	0.09	0	0	27.0
	6	2.05	3.72	72.8	171.4
10.10.85	1	0	0	0	11.7
	2	0.03	0	0	47.5
	3	0.14	0	0	24.3
	4	0.07	0	15.3	70.7
	5	0.8	0	21.5	104.1
	6	1.78	0.92	14.4	144.5
07.11.85	1	0.76	3.99	180.4	80.9
	2	0.82	0	0	0
	3	0.06	1.71	22.5	59.2
	4	0	0.78	0	49.1
	5	0	0	0	0
	6	1.06	5.87	0	75.1
05.12.85	1	0.11	0	0	16.6
	2	0.08	0	46.0	12.9
	3	0.03	0	0	0
	4	0	0	0	30.9
	5	0	0	0	0
	6	0.79	3.32	0	0
02.01.86	1	0	0	0	2.9
	2	0.03	0	0	8.3
	3	0.13	0	0	46.6
	4	0.15	0	0	1.9
	5	0.32	0	0	2.1
	6	3.83	8.36	28.6	181.6
23.03.86*	1	0	3.2	0	0
	2	0.26	2.38	0	0
	3	0.63	5.47	0	11.7
	4	0.64	4.28	0	16.9
	5	1.62	13.88	19.8	79.8
	6	10.47	83.20	490.9	628.9

Table A5.1 contd

COLLECT. DATES	SITE	CADMIUM	COPPER	LEAD	ZINC
MEAN	1	0.26	2.74	63.5	21.8
	2	0.18	1.5	59.2	25.8
	3	0.28	1.89	85.7	37.7
	4	0.19	1.94	56.6	29.0
	5	0.4	2.67	105.6	47.1
	6	3.03	17.77	148.7	213.7

* Moss bags left out for a period of 11 weeks.

APPENDIX 6.

Table A6.1

CONTENTS OF MANCHESTER TÜLLGRENS. TOTALS FROM 4 X $0.25M^2$ QUADRATS (Mites & Collembola only.)

	HAW WOOD	PEGWELL WOOD	TOCKINGTON PARK	KINGTON GROVE	KNAPP WOOD	WETMOOR WOOD
Dry Weight (g)	342	261	432	370	386	193
Volume of Sample (l)	12	9	8	9	17	10
% of Whole Leaves	20	48	20	80	73	94
% of Broken Leaves	50	35	50	15	20	2
Twigs	30	7	30	5	7	4
Grass		10				
Damaeus onustus	284	291	190	329	252	
Box Mites	15	14	380	304	191	196
Other Cryptostigmata	1117	190	3531	2570	1446	1367
Mesostigmata	1811	532	2431	3665	1951	2032
Sminthuridae	0	0	22	0	18	116
Other Collembola	1730	569	847	284	511	800
Total Mites	3227	1027	6532	6867	3840	3595
Total Collembola	1752	569	869	284	529	916
TOTAL	4979	1596	7401	7151	4369	4511

Table A6.2

NUMBERS OF ANIMALS DETAINED FROM THE SOIL CORES EXTRACTED AT YORK

Cores were divided into 3cm sections labelled top (T), middle (M) and bottom (B) in the table. The soil at some sites was not deep enough for all three layers. A, B, C and D are the four replicas.

SITE	SECTION	REPLICATE	DRY WT. (g)	OPPIA SPP	OTHER CRYPTO STIGMATA	MESO-STIGMATA	SMINTHUR-IDAE	OTHER COLLEMBOLA	COLEOPTERA LARVAE	DIPTERA LARVAE	HOMOPTERA	CHILOPODA	DIPLODA	ISOPODA	PSEUDOSCOR-PIONES	MOLLUSCA	LUMBRICIDAE	TOTAL MITES	TOTAL COLLEMBOLA
WM	T	A	87.92	57	21	30		12			1	1						108	12
WM	T	B	97.61	5	3	16		4		1								24	4
WM	T	C	71.02	5	11	8		2										24	2
WM	T	D	80.46	2	13	2	1	2										17	3
KW	T	A	73.63		3	2							1					5	0
KW	B	A	163.73		1				1									1	0
KW	T	B	95.65	3	7	3		6		2		2						13	6
KW	B	B	107.18		1													1	0
KW	T	C	78.6	5	6	11	1	12										22	13
KW	B	C	133.67	1		1		1										2	1
KW	T	D	93.63	7	1	12		1	1	1		4	1	2	1			20	1
KG	T	A	38.15	6	1	3		16	1			1						10	16
KG	B	A	89.79					1										0	1
KG	T	B	23.87		30	29			1	4		1	5	1				59	0
KG	M	B	39.11	6	9	11		2		7								26	2
KG	B	B	99.88					1										0	1
KG	T	C	18.61		1	18		1	3									19	1
KG	M	C	78.48	2	1	10		8	1	1							3	13	8

Table A6.2 contd

SITE	SECTION	REPLICATE	DRY WT. (g)	OPPIA SPP.	OTHER CRYPTO STIGMATA	MESO-STIGMATA	SMINTHUR-IDAE	OTHER COLLEMBOLA	COLEOPTERA LARVAE	DIPTERA LARVAE	HOMOPTERA	CHILOPODA	DIPLOPODA	ISOPODA	PSEUDOSCOR-PIONES	MOLLUSCA	LUMBRICIDAE	TOTAL MITES	TOTAL COLLEMBOLA
KG	B	C	130.07		2			1										2	1
KG	T	D	13.22	1	18	27		12		1		4					2	55	12
KG	M	D	45.13	1	4	4		7	1				1					9	7
KG	B	D	94.9		1				2									1	0
TP	T	A	55.26		8	8		10	1									16	10
TP	B	A	104.78		3			2	1									3	2
TP	T	B	32.69	5	17	40	1	18	9		1	1				1	1	62	18
TP	B	B	91.21			7	1	1	1	1								7	2
TC	T	C	11.56		13	8		3		2		2	2					21	3
TP	M	C	30.9	3	1	24		7		2		4	1					28	7
TP	B	C	58.83					1										20	1
TP	T	D	48.66	5	25	31	2	36		6			1					61	38
TP	B	D	102.78			2		1		1							1	2	1
PW	T	A	42.05	58	16	27		6				7		2	2	1			
PW	B	A	112.23	7		6				1									
PW	T	B	72.03	8	5	4		6	3	10									
PW	B	B	116.61	2		1			2										
PW	T	C	46.66	6	7	7	2	9	6			1							
PW	M	C	91.66		2	1		1		1		1						3	1
PW	B	C	142.44															0	0
PW	T	D	17.27		1	3											3	4	0

Table A6.2 contd

SITE	SECTION	REPLICATE	DRY WT (g)	OPPIA SPP.	OTHER CRYP-TOSTIGMATA	MESO-STIGMATA	SMINTHUR-IDAE	OTHER COLLEMBOLA	COLEOPTERA LARVAE	DIPTERA LARVAE	HOMOPTERA	CHILOPODA	DIPLOPODA	ISOPODA	PSEUDOSCOR-PIONES	MOLLUSCA	LUMBRICIDAE	TOTAL MITES	TOTAL COLLEMBOLA
PW	M	D	70.46		2	16	1	32	1									18	33
PW	B	D	118.96			1		1										1	1
HW	T	A	14.82	60	4	38		61		1		2		2				102	61
HW	M	A	27.95		2	4				5								6	0
HW	B	A	83.49							1								0	0
HW	T	B	15.28	66	13	39		30	4									118	30
HW	M	B	28.31	25	2	7		5				1	1					24	5
HW	B	B	56.27	1		1												2	0
HW	T	C	5.23	18	9	14				1		3		3				41	0
HW	M	C	36.93	21	5	21		7		3								47	7
HW	B	C	85.84			6		4										6	4
HW	T	D	17.66	89	6	28		7				3						123	7
HW	M	D	25.74		1	3			1									4	0
HW	B	D	40.47	5		1												6	0

APPENDIX 7.

Ophyiulus pilosus adults.

A smaller number of O. pilosus were collected from Knapp wood and were treated identically to the T. niger specimens (see section 5.3 for details). Unfortunately this species proved more difficult to maintain in laboratory conditions and by the termination of the experiment all were dead. Table A6.1 shows the results for O. pilosus. It appears that a similar situation to that in T. niger occurs in this species. Metal concentrations in the whole animals are very similar whereas there are bigger differences between concentrations in the faeces. Faeces production is also greater in the Wetmoor fed animals. However the standard errors are very large and none of these differences are significant. Obviously the number of animals used was very small. Repeating this experiment with more replicates of both species and larger numbers of each sex would help clarify the situation.

Table A7.1

METAL CONCENTRATIONS IN ANIMALS AND FAECES AND t-TESTS BETWEEN THEM FOR ADULT

OPHYIULUS PILOSUS FED ON DIFFERENT LITTER TYPES

		ANIMALS FED ON WETMOOR LEAVES	ANIMALS FED ON HAW LEAVES	t-TEST	d.f.	p
Animals	n	2	4			
	Cd	3.65 ± 3.16	2.86 ± 1.45	0.23	1.4	0.86 ns
	Cu	405.8 ± 49.3	398.7 ± 17.5	0.14	1.3	0.91 ns
	Pb	0.94 ± 0.06	13.7 ± 11.2	-1.14	3.0	0.34 ns
	Zn	189.1 ± 24.6	242.2 ± 27.1	-1.45	3.3	0.24 ns
Mean Dry Wt.Faeces (g)		9.895 ± 0.995	1.747 ± 0.524	7.25	1.6	0.087 ns
Mean Faeces per day(g)		1.345 ± 0.875	0.214 ± 0.0836	1.29	1.0	0.42 ns
Faeces	n	4	8			
	Cd	1.82 ± 0.33	19.1 ± 11.1	-1.55	7.0	0.17 ns
	Cu	152.4 ± 44.2	391.0 ± 195.0	-1.19	7.7	0.27 ns
	Pb	26.58 ± 6.63	269.0 ± 137.0	-1.77	7.0	0.12 ns
	Zn	212.3 ± 27.6	1195.0 ± 491.0	-2.0	7.0	0.086 ns

APPENDIX 8.

METAL CONCENTRATIONS IN NEWTS.

Newts were caught in the pitfall traps at two sites, Haw and Tockington Park. The majority were smooth newts, Triturus vulgaris but one from each site was T. helveticus the palmate newt. Metal concentrations in each animal were determined using methods previously described. The results are given in Table A7.1.

For all four metals the mean concentrations in the animals from Haw are higher than those from Tockington Park. Using t-tests on log transformed data the differences in cadmium and lead are shown to be significant, despite the low number of replicates.

Very little data is available on the concentrations of metals in amphibians. Beyer et al. (1985) records that levels in frogs, toads and salamanders were fairly low 10km upwind of a smelting works, comparable to those of mice and voles and lower than shrews. However amphibians were rare at a closer site downwind from the pollution source and were not sampled. Fangmeier & Steubing (1986) detected cadmium in the kidneys and lead in the bone of a frog species from an unpolluted site. Ireland (1977b) observed uptake of lead in toads fed on contaminated worms.

Avery et al. (1983) found very low levels of copper, cadmium, lead and zinc in lizards from beside roads,

Table A8.1

METAL CONCENTRATIONS (μgg^{-1} DRY WEIGHT)

IN NEWTS FROM TWO SITES

	HAW WOOD	TOCKINGTON PARK	t	df	p
n	4	8			
Cd	21.41 ± 7.96	2.31 ± 0.38	-5.09	5.5	0.01
Cu	29.49 ± 6.52	23.03 ± 7.95	-1.44	9.9	ns
Pb	79.1 ± 17.6	27.32 ± 6.1	-3.59	8.1	0.01
Zn	357.5 ± 79.5	239.9 ± 25.7	-1.45	4.1	ns

t-tests performed on log transformed data.

significantly lower than many of their potential food supply at the same sites. It might be suggested that amphibians seem to accumulate lead and cadmium to a greater extent than reptiles.

APPENDIX 9.

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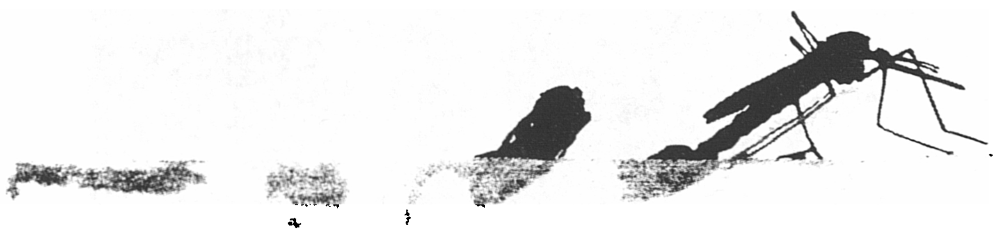

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THE EFFECTS OF HEAVY METAL POLLUTION ON WOODLAND COMMUNITIES OF SURFACE ACTIVE CARABIDAE (COLEOPTERA).

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Introduction

Relatively few studies have investigated the effects of heavy metal pollution on the ecology of invertebrate communities. Those invertebrates studied include the Oligochaeta (eg. Helmke et al. 1979), Isopoda (Hopkin & Martin 1984a) and Chilopoda (Hopkin & Martin 1984b). This work considers the Carabidae, an important group of soil animals which rarely have been investigated from this view point.

The Avonmouth area of South West England has, for many years, been used for studies of pollution (Coughtrey et al. 1979). The chief source of pollutants is a large smelting works which emits zinc, lead, cadmium and copper. These pollutants are carried by the prevailing winds, mainly in a north easterly direction (Gill et al 1975). Coughtrey et al. (1979) have shown that woodlands down wind of the smelter are heavily polluted and show features such as greatly increased litter layers. Hopkin et al. (1985) have commented on the differences of the soil and litter fauna from these woods.

Methods

Carabids were collected from six woodlands each containing a mixture of Oak, Ash, Hazel and Hawthorn. These lie approximately north east of the Avonmouth smelter, which is 3 km from the nearest site and 23 km from the furthest. In each wood 15 plastic pitfall traps (each 8 cm diameter by 11 cm deep and containing a formalin detergent mixture) were laid 2 m apart in a 5 x 3 grid. The traps were emptied fortnightly from 11.iv.85 to 19.xii.85 and the Carabidae collected were sorted and identified. Samples of leaf litter (15 replicates) were taken at random from every site during February 1986. These were analysed for zinc, lead, cadmium and copper using flame atomic absorption spectrophotometry with a Varian AA 775.

Metal concentrations were correlated with aspects of the carabid fauna including numbers of individuals, numbers of species, diversity and distributions of capture in relation to time. Two tailed Spearman rank correlation coefficients were used to examine these data and the results were grouped using the combined probability analysis developed by Fisher (Sokal & Rohlf 1969) and hereafter called F.C.P.

Results.

A total of 3373 individuals of 38 species of Carabidae were examined. The breakdown of these across the sites is listed in Table 1 as are the distances from the smelting works and the concentrations of metals found in the litter, humus and soil layers. Using a nonparametric measure of concordance (Meddis 1984) it can be seen that there is the same degree of change of metal concentration throughout the sites ($H=19.4$, $df=5$, $p=0.002$).

With Spearman rank correlation coefficients no significant correlations were found between either numbers of individuals or numbers of species of Carabidae, and the concentration of any of the metals in the litter layer. Data were combined using combined probability tests but the results were again insignificant (Table 2a).

When the diversity of the carabid fauna was investigated (using Shannon's diversity index $\bar{H}'$ - Pielou 1975) again no significant correlations were found with metal concentration. Using combined results an overall influence on diversity was found with metal concentration (X^2_8=17.96, p=0.022). Although total numbers of individuals and species are not affected by metal concentrations, since they are components of the diversity index used, they may still be involved in this effect. It may be that captures over the year are affected by distance from the smelter. Median capture of total individuals (ie. the date on which the median individual was captured) was found to be negatively correlated with distance from the smelter (Rs=-0.870, p=0.024). The two most abundant and widespread species found during this study (Nebria brevicollis and Abax parallelepipedus) were similarly tested and N. brevicollis showed a similar relationship to total Carabidae (Rs=-0.879, p=0.021). However, median capture of A. parallelepipedus was not correlated with distance from the smelter (Rs=-0.676, p=0.14).

The median captures were analysed with respect to metal concentration and in all cases the overall effect was of later median with increased pollution (Table 2b). This effect is not simply one of increased numbers of autumn breeding species being found nearer to the smelter, since there is no significant difference between the numbers of spring, summer and autumn breeders found at the sites (X^2_{10}=3.05, p=0.98). However there is a negative correlation between the numbers of individuals belonging to spring breeding species and metal concentration (Table 2c) and a corresponding positive correlation between numbers of individuals from autumn breeding species and metal concentration. The latter case appears to be at least partially explained by the numbers of N. brevicollis occurring since this autumn breeder shows a strong overall positive correlation with metal concentration (F.C.P. X^2_8=26.38, p=0.001) and analysis of the numbers of individuals from autumn breeding species without N. brevicollis shows a reduced relationship (F.C.P. X^2_8=20.11, p=0.011).

Discussion.

Changes in the community structure of Carabidae have been recorded by Lesniak (1980) and appeared to involve the reduction of rarer species and the rise in importance of dominant ones with increasing pollution. During the present study, although the proportions of spring and autumn breeding species are unaffected by metal pollution, the relative numerical importance of each breeding type are related. This and the delay in time of median capture in relation to metal pollution of the two widespread species, seems to point to an overall inhibition of activity earlier in the season in areas of high metal concentration. This may be the result of pollution affecting the food supply. Many Carabidae are surface active predators and some (eg. Nebria brevicollis) may undergo diapause during the summer in order to avoid the paucity of prey species at this time (Penney 1969). In addition the onset of diapause in this species appears to be initiated by high bodily fat reserves and is, therefore dependent on the food availability during spring and early summer. Metal pollution has been shown to decrease micro- arthropod numbers (Strojan 1978) and may

Table 1 Site characteristics

	Haw Wood	Pegwell Wood	Tockington Park	Kington Grove	Knapp Wood	Wetmoor
Distance from smelter (km)	3.0	7.8	11.6	13.9	18.8	23.0
No. of species of Carabidae	19	23	20	18	22	21
No. of individuals of Carabidae	630	968	493	515	250	517
Species diversity	1.88	1.43	2.23	1.90	2.40	1.94
Zn in litter (ug/g)	1389.78	257.03	193.77	159.18	112.84	74.45
Pb in litter (ug/g)	1142.42	137.94	125.67	97.95	49.98	32.84
Cd in litter (ug/g)	19.88	3.30	1.68	2.05	1.01	0.60
Cu in litter (ug/g)	151.41	23.94	17.64	18.17	13.68	12.26

Table 2 The overall effect of heavy metal concentration on aspects of carabid communities. Combined probabilities were calculated from the probabilities of Spearman rank correlation coefficients of the dependant variables against individual metal concentrations in the leaf litter. The sign in parentheses indicates the direction of the coefficients.

	Combined probabilities.	
	X^2_8	p
a. Site characteristics.		
No. of individuals	11.58	0.172 (+)
No. of species	2.85	0.943 (-)
Diversities	17.96	0.022 (-)
b. Median captures.		
Total species	34.23	<0.001 (+)
Nebria brevicollis	19.19	0.015 (+)
Abax parallelepipedus	25.51	0.002 (+)
c. Number of individuals of various breeding types.		
Spring breeders	26.38	0.001 (-)
Autumn breeders	37.05	<0.001 (+)

increase the available refuges from predation for these reduced numbers (eg. by the increased depth of leaf litter described by Coughtrey et al. (1979)). This may result in delayed diapause in those areas of high pollution. For species which unlike N. brevicollis do not have an obligatory diapause (eg. Abax parallelepipedus (Thiele 1977)) the effect of low prey species numbers may simply delay peak activity time.

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APPENDIX 10.

Environmental Pollution 48 (1987) 61–76

Aspects of the Ecology of Carabidae (Coleoptera) from Woodlands Polluted by Heavy Metals

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ABSTRACT

The carabid faunas of six woodlands at varying distances from a source of heavy metal pollution were studied. Concentrations of zinc, lead, cadmium and copper were determined in three surface layers at each wood. Whereas no gross effects of pollution on populations were identified, some differences relating to the ecology and life histories of the animals were found. The numbers of individuals and numbers of species of Carabidae at each site were not significantly correlated to metal concentration. However, species diversities (Shannon Weiner H') were. Later dates of median capture of total Carabidae were found in the sites nearest to the pollution source. This also occurred with a common species, Nebria brevicollis. *At the most polluted site this species also showed an absence of summer diapause. This may be related to scarcity of prey. Negative correlations of numbers of spring breeders with metal concentration but positive correlations of autumn breeders were found in all the soil layers. The sites were significantly different in the relative distributions of individuals in each of four size categories.*

INTRODUCTION

Heavy metal pollution has been shown to have an effect on a number of terrestrial invertebrate groups, including oligochaetes (e.g. Helmke *et al.*

Environ. Pollut. 0269-7491/87/$03·50

1979), isopods (Joosse *et al.*, 1981; Hopkin & Martin, 1984*a*) and chilopods (Hopkin & Martin, 1984*b*). Such studies have tended to concentrate on the effect on individual species rather than on communities of animals and have examined mainly physiological, rather than ecological, effects.

Workers in pollution studies have for many years used the Avonmouth area of Southwest England (Coughtrey *et al.*, 1979) as a study area. The prevailing winds in this area result in heavy depositions of zinc, lead, cadmium and copper emitted from a large lead–zinc smelting works, mainly to the north-east (Gill *et al.*, 1975). Woodlands downwind of this industrial site have been regarded as heavily polluted (Martin *et al.*, 1982) and to have greatly increased litter depths (Coughtrey *et al.*, 1979). Hopkin *et al.* (1985) also noted differences in the soil and litter fauna.

Carabidae are common, mainly nocturnal predators found in large numbers in woodlands. They exhibit a wide range of sizes and food preferences. These animals are active from early spring to late autumn, with peak activity occurring during the breeding seasons, which may be in the spring, summer or autumn, depending on the species.

The present study examines the communities of Carabidae in woodlands at a range of distances from the smelting works and investigates the relationships between degree of metal pollution and the ecology of this important group of predators from woodlands.

METHODS

Carabidae were sampled from six deciduous woodlands, in the Avonmouth area, extending in a line approximately north-east from the smelting works. Each woodland had an upper canopy which included *Quercus* spp. together with *Fraxinus excelsior*, a lower canopy containing *Crataegus monogyna*, and usually *Corylus avellana* and *Acer campestre*, and undergrowth of *Rubus fruticosus* agg. with, in four sites, *Endymion non-scriptus*. Litter depth was measured at each site in March 1986 and random samples of organic and mineral soil layers were taken in February 1986 for pH determination and heavy metal analysis. For the latter, samples were dried at 70°C before being ground into powder. Aliquots of 0·5 g were digested in 10 ml of boiling Analar grade concentrated nitric acid. Each sample was analysed for zinc, lead, cadmium and copper using atomic absorption spectrophotometry with background correction. The soil layers sampled were the leaf litter layer (L), the combined fermentation and humidification layers (F/H) and the upper 5 cm of the mineral soil layer (soil).

In each woodland, a grid of 15 plastic pitfall traps (each 8 cm in diameter and 11 cm deep) was laid in a 5 × 3 formation. The traps contained 25 ml of

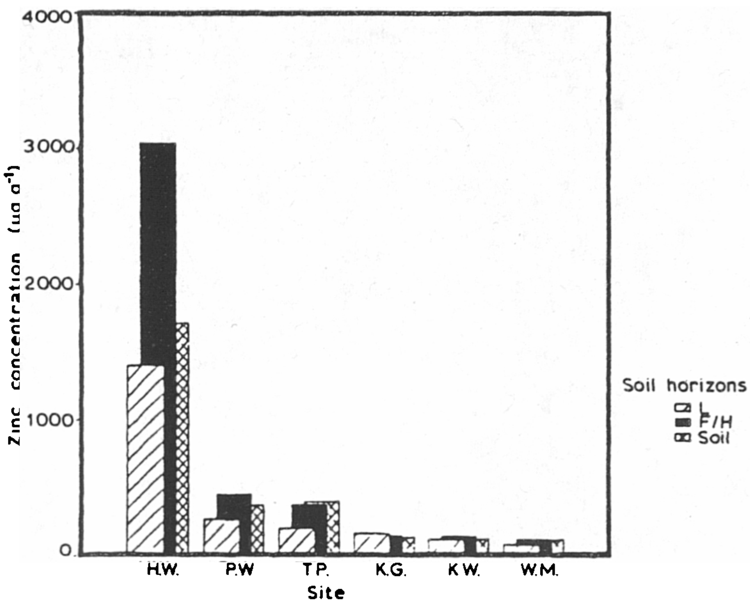
4% formalin solution with a drop of detergent added to reduce surface tension and were 2 m apart. The sites were visited fortnightly from April to December during 1985 and the contents of the traps removed. The animals collected were sorted and identified. Whilst such sampling techniques are not necessarily the best for sampling all Carabid populations, the methods adopted were strictly comparable in all six woodlands and the results were treated on this comparative basis. Previous workers (e.g. Bengtsson & Rundgren, 1984) have also noted that when the numbers and sizes of the pitfall traps and duration of the sampling period are the same at each site, the data are comparable. It was therefore possible to investigate the relationships between the Carabidae and metal concentrations in the series of woodlands by using two-tailed Spearman rank correlation coefficients. These results were then combined using the combined probability analysis developed by Fisher, hereafter called FCP (Sokal & Rohlf 1969). In every case where a significant value was obtained for FCP, the Spearman rank correlation coefficients had consistent signs and these were taken as the direction of the relationship. Such significant results imply an association which requires interpretation though, of course, they cannot be taken as proof of a direct relationship.

RESULTS

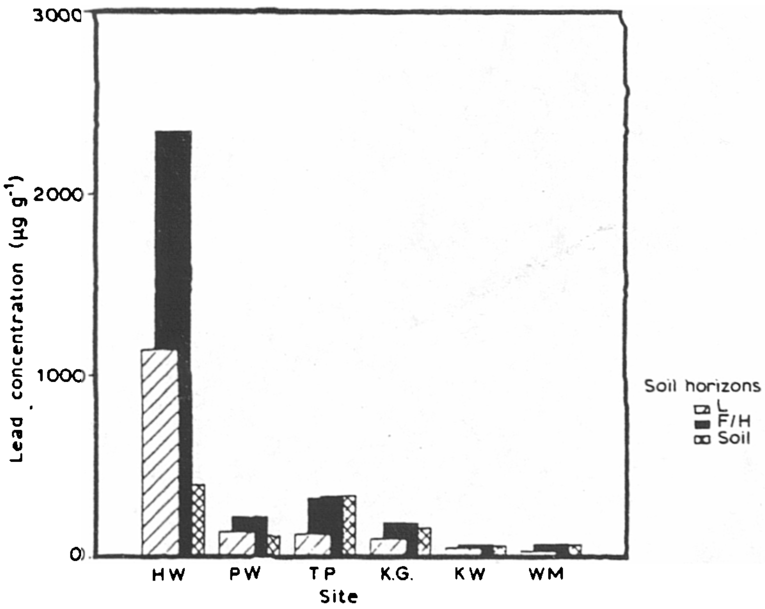
Litter depth decreased with distance from the smelting works (Table 1). The pH values in the soil levels (Table 1) were fairly stable, ranging from 4·0 to

TABLE 1
Characteristics of the Sites

Site	*Distance from smelter (km)*	*Grid reference*	*Mean litter depth (mm)*	*Litter*	*pH F/H*	*Mineral soil*
Haw Wood HW	3·0	ST 558 880	131·0	4·85	4·65	5·5
Pegwell Wood PW	7·8	ST 593 826	85·6	4·9	4·3	4·9
Tockington Park TP	11·6	ST 624 858	44·0	5·05	4·05	4·0
Kington Grove KG	13·9	ST 622 893	86·0	4·4	3·45	3·55
Knapp Wood KW	18·8	ST 665 916	61·0	5·4	4·85	5·35
Wetmoor WM	23·0	ST 743 874	32·0	5·2	5·05	5·2

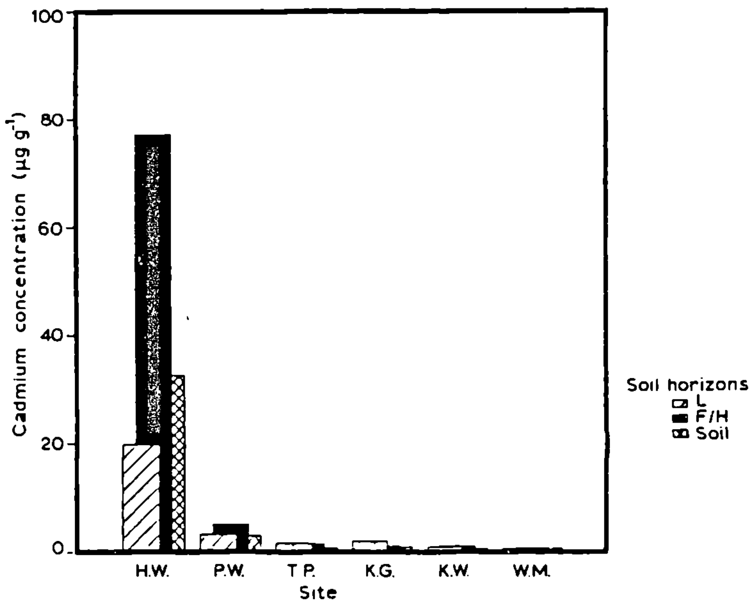


(a)



(b)

Fig. 1. Heavy metal concentrations of the organic and mineral soil layers at the sampling sites (for site abbreviations see Table 1).



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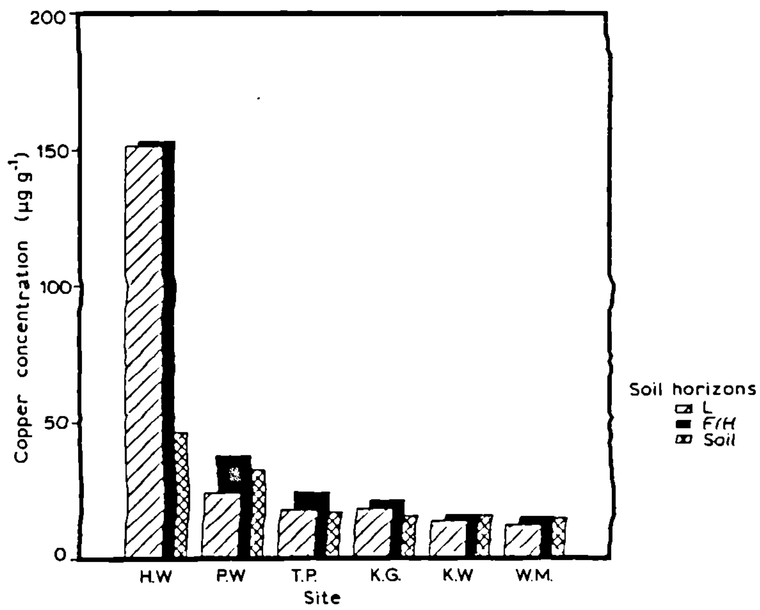

(d)

Fig. 1.—*contd.*

TABLE 2
Carabidae Found at the Sites

Sites	*WM*	*KW*	*KG*	*TP*	*PW*	*HW*
Cychrus caraboides (Linnaeus)	3					87
Carabus granulatus Linnaeus	32					
Carabus nemoralis Muller						6
Carabus violaceus Linnaeus	1			10	1	52
Leistus ferrugineus (Linnaeus)	8	46	48	55	35	24
Leistus fulvibaris Dejean	2	1			21	16
Leistus rufomarginatus (Duftschmid)	1					
Nebria brevicollis (Fabricius)	9	34	27	97	487	152
Notiophilus biguttatus (Fabricius)	16	7	9	30	7	1
Notiophilus rufipes Curtis	2		1			
Loricera pilicornis (Fabricius)		2	20	7	2	4
Clivina fossor (Linnaeus)					1	
Bembidion litorale (Olivier)	4	1			1	
Bembidion tetracolum Say	1					
Bembidion guttula (Fabricius)	19	11	17	7	6	7
Pterostichus cupreus (Linnaeus)	9	12	3	11	3	
Pterostichus madidus (Fabricius)	95	34	13	84	49	2
Pterostichus melanarius (Illiger)	10	8	39	6	5	
Pterostichus niger (Schaller)				1	10	
Pterostichus nigrita (Paykull)		1				
Pterostichus stenuus (Panzer)	9	15	3	7	2	17
Pterostichus versicolor (Sturm)				1		1
Abax parellelepipedus (Piller & Mitterpacher)	192	45	265	104	310	228
Calathus melanocephalus (Linnaeus)			4	1	7	2
Calathus piceus (Marsham)			17			
Platyderus ruficollis (Marsham)		19		5	4	1
Agonum albipes (Fabricius)	1		7		3	24
Agonum assimile (Paykull)	101		19			4
Agonum dorsale (Pontoppidan)		3		1		
Agonum obscurum (Herbst)		2				
Agonum viduum (Panzer)		1				
Amara familiaris (Duftschmid)	1	2	4		4	
Amara similata (Gyllenhal)	1	3	15	16	5	1
Harpalus rufipes (Degeer)		1	4	47	1	
Bradycellus harpalinus (Serville)					1	
Dromius linearis (Olivier)				1		
Dromius quadrimaculatus (Linnaeus)		1		2	3	1
Metabletus foveatus (Fourcroy)		1				
Number of individuals	517	250	515	493	968	630
Number of species	21	22	18	20	23	19
Species diversity (H')	1·94	2·40	1·90	2·23	1·43	1·88

Key to sites: WM, Wetmoor; KW, Knapp Wood; KG, Kington Grove; TP, Tockington Park; PW, Pegwell Wood; HW, Haw Wood.

5·5, with the exception of Kington Grove which showed more acidity in all the soil layers. Metal concentrations (Fig. 1) decreased with distance from the smelter and had a hierarchy of concentrations of zinc, lead, copper and then cadmium. At most of the sites the F/H layer showed the highest concentrations of heavy metals. The concentrations of different metals at each site were analysed using a non-parametric measure of concordance (Meddis, 1984) and this showed that the same degree of change occurred throughout the sites with the different metals (H = 19·4, df = 5, $p = 0{\cdot}002$).

Some 3373 individuals of 38 species of Carabidae were identified and examined (Table 2). No significant correlations were found between the numbers of individuals or the numbers of species of beetles and the metal concentrations at any of the surface layers, with the exception of cadmium at the soil layer, which was positively correlated with numbers of individuals (Table 3). With combined data, the results were non-significant, indicating that the single significant result may have occurred by chance. The diversities

TABLE 3

Correlations Between Metal Concentration and Numbers of Individuals, Numbers of Species and Species Diversity of Carabidae

		Number of individuals		*Number of species*		*Diversities*	
		Rs	*p*	*Rs*	*p*	*Rs*	*p*
L layer							
Zn		0·540	0·266	−0·142	0·787	−0·657	0·156
Pb		0·540	0·266	−0·142	0·787	−0·657	0·156
Cd		0·600	0·208	−0·257	0·623	−0·771	0·072
Cu		0·600	0·208	−0·257	0·623	−0·771	0·072
FCP	χ^2_8	11·58		2·85		17·96	
L layer	p	0·172		0·943		0·022	
F/H layer							
Zn		0·540	0·266	−0·142	0·787	−0·657	0·156
Pb		0·486	0·329	−0·371	0·468	−0·543	0·266
Cd		0·540	0·266	−0·142	0·787	−0·657	0·156
Cu		0·540	0·266	−0·142	0·787	−0·657	0·156
FCP	χ^2_8	10·17		2·96		13·80	
F/H layer	p	0·254		0·937		0·088	
Soil layer							
Zn		0·540	0·266	−0·142	0·787	−0·657	0·156
Pb		0·314	0·544	−0·257	0·623	−0·429	0·397
Cd		0·886	0·019	−0·257	0·623	−0·943	0·005
Cu		0·429	0·397	−0·086	0·872	−0·484	0·329
FCP	χ^2_8	13·64		2·65		18·38	
Soil	p	0·093		0·954		0·019	

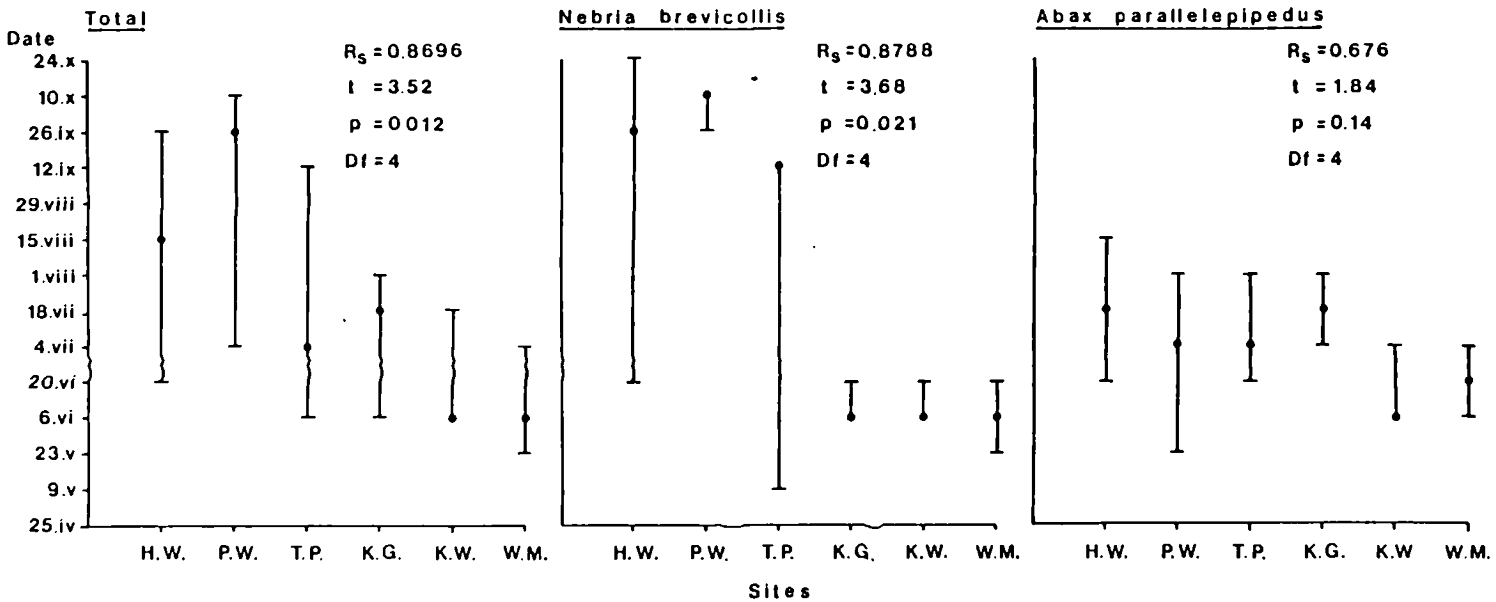

Fig. 2. Date of median capture of Carabidae at the sampling sites indicating interquartile ranges and correlation coefficients between date of median capture and the distance from the pollution source. For sample sizes see Table 1.

of Carabidae caught at each site were calculated using Shannon's diversity index H′ (Pielou, 1975). These were analysed with respect to metal concentrations and again, only the relationship with the cadmium concentration of the soil layer was significantly correlated. Here the result was a negative correlation (Table 3). When the results for diversities were combined, negative correlations were found between diversity and metal concentration at the L layers and the soil layers (Table 3). Neither pH nor litter depth showed significant correlations with numbers of individuals, numbers of species, or the diversities of Carabidae at the sites.

The distributions of captures over the year were significantly different in the different sites ($\chi^2_{85} = 1327, p < 0{\cdot}00001$). The dates of median capture of individuals of total Carabidae (measured as the date on which the median animal was caught) were negatively correlated with distance from the pollution source ($Rs = -0{\cdot}870$, $p = 0{\cdot}024$, see Fig. 2). Two species (*Nebria brevicollis* and *Abax parallelepipedus*) were found to be present in large numbers at all of the sites and the date of median capture of *N. brevicollis* is negatively correlated with the distance from the smelting works ($Rs = -0{\cdot}879$, $p = 0{\cdot}021$). This species shows an absence of its normal summer diapause (Penney, 1969) at Haw Wood, the site closest to the smelter (Fig. 3). The date of median capture of *A. parallelepipedus* was not

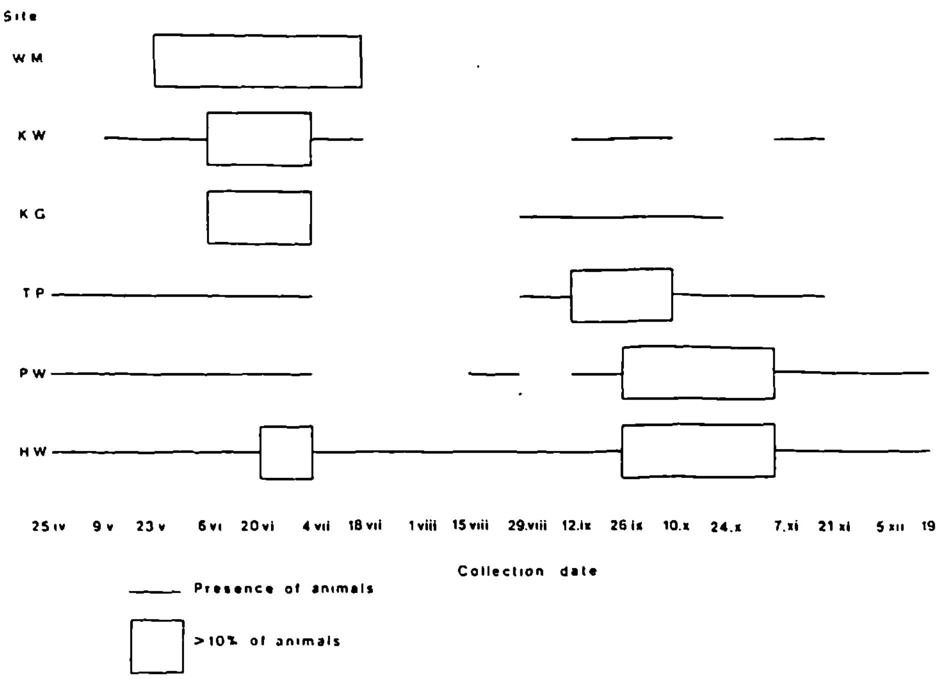

Fig. 3. Activity of *Nebria brevicollis* over the sampling period at each site, as measured by pitfall sampling.

significantly correlated with distance from the smelter ($Rs = -0{\cdot}676$, $p = 0{\cdot}14$).

When the dates of median capture were analysed with metal concentrations, in all cases (i.e. *N. brevicollis*, *A. parallelepipedus* and total Carabidae) the overall effects were of later medians with increased metal concentration (Table 4). This does not seem to be due simply to increased

TABLE 4

Correlations Between Metal Concentration and Dates of Median Capture of Carabidae, *Nebria brevicollis* and *Abax parallelepipedis*

		Total Carabidae		N. brevicollis		A. parallelepipedis	
		Rs	*p*	*Rs*	*p*	*Rs*	*p*
L layer							
Zn		0·870	0·024	0·879	0·021	0·676	0·140
Pb		0·870	0·024	0·879	0·021	0·676	0·140
Cd		0·928	0·008	0·758	0·081	0·794	0·059
Cu		0·928	0·008	0·758	0·081	0·794	0·059
FCP	χ^2_8	34·23		25·51		19·19	
L layer	p	<0·001		0·002		0·015	
F/H layer							
Zn		0·870	0·024	0·879	0·021	0·676	0·140
Pb		0·696	0·125	0·758	0·081	0·735	0·076
Cd		0·870	0·024	0·879	0·021	0·676	0·140
Cu		0·870	0·024	0·879	0·021	0·676	0·140
FCP	χ^2_8	26·54		28·21		16·95	
F/H layer	p	0·001		0·001		0·031	
Soil layer							
Zn		0·870	0·024	0·879	0·021	0·676	0·140
Pb		0·580	0·228	0·515	0·296	0·853	0·031
Cd		0·841	0·036	0·636	0·124	0·765	0·077
Cu		0·725	0·103	0·879	0·021	0·412	0·417
FCP	χ^2_8	21·61		22·06		17·76	
Soil	p	0·006		0·005		0·024	

numbers of autumn breeding species being found near to the smelter, since no significant difference was found between the numbers of species breeding in the spring, summer or autumn at the sites ($\chi^2_{10} = 3{\cdot}05$, $p = 0{\cdot}98$, see Table 5). There are, however, negative correlations between the numbers of spring breeding individuals and the concentrations of metals found at the sites (Table 6). In addition, there are positive correlations of numbers of autumn breeding individuals with metal concentrations. The effect on autumn breeders may be due, in part, to the influence of *N. brevicollis*. The numbers of this autumn breeding species show strong positive correlations with metal concentration (FCP: L $\chi^2_8 = 26{\cdot}38$, $p = 0{\cdot}001$; F/H $\chi^2_8 = 27{\cdot}50$, $p < 0{\cdot}001$; soil $\chi^2_8 = 22{\cdot}59$, $p = 0{\cdot}005$). Analysis of the numbers of autumn breeding individuals without this species indicates that there is a significant correlation only with metal concentrations in the L layer (FCP: L $\chi^2_8 = 20{\cdot}11$, $p = 0{\cdot}011$; F/H $\chi^2_8 = 13{\cdot}80$, $p = 0{\cdot}088$; soil $\chi^2_8 = 13{\cdot}00$, $p = 0{\cdot}113$).

The species found at the sites were allocated size categories, with similar numbers of species being placed in each of four groups (Table 5). The sites

TABLE 5
Breakdown of Species According to Breeding Seasons and Size

	Spring breeders	*Summer breeders*	*Autumn breeders*
Size I > 12·5 mm	*Carabus granulatus* *Carabus nemoralis*	*Cychrus caraboides* *Pterostichus madidus* *Abax parallelepipedus*	*Carabus violaceus* *Pterostichus melanarius* *Pterostichus niger* *Harpalus rufipes*
Size II 7·5–12·5 mm	*Pterostichus cupreus* *Pterostichus nigrita* *Pterostichus versicolor* *Agonum assimile* *Amara similata*	*Agonum viduum*	*Leistus rufomarginatus* *Nebria brevicollis* *Calathus piceus* *Agonum albipes*
Size III 6·0–7·4 mm	*Loricera pilicornis* *Clivina fossor* *Pterostichus strenuus* *Platyderus ruficollis* *Agonum dorsale* *Amara familiaris*	*Notiophilus rufipes*	*Leistus ferrugineus* *Leistus fulvibarbis* *Calathus melanocephalus*
Size IV < 6·0 mm	*Bembidion litorale* *Bembidion guttula* *Metabletus foveatus*	*Notiophilus biguttatus* *Bembidion tetracolum* *Agonum obscurum* *Dromius linearis* *Dromius quadrimaculatus*	*Bradycellus harpalinus*

were found to have significantly different proportions of individuals belonging to these size groups ($\chi^2_{15} = 501$, $p = <0{\cdot}00001$). No significant correlation was found between the median size of the animals at the sites and the metal concentration (FCP: L $\chi^2_8 = 2{\cdot}04$, $p = 0{\cdot}979$; F/H $\chi^2_8 = 1{\cdot}64$, $p = 0{\cdot}989$; soil $\chi^2_8 = 5{\cdot}50$, $p = 0{\cdot}705$). The proportions of individuals in each size category were analysed against metal concentration and, whereas no significant relationships were found with sizes I and III, the proportions of individuals from size II were found to be positively correlated with the metal concentrations in the F/H layers, and those for size IV were negatively correlated with the metal concentrations at all the soil layers (Table 7).

Of the size II individuals, 75·6% were *N. brevicollis*. The results found for this size group could be due to the effect of this species. Removal of *N.*

TABLE 6
Correlations Between Metal Concentrations and Numbers of Spring and Autumn Breeding Individuals

		Spring breeders		*Autumn breeders*	
		Rs	*p*	*Rs*	*p*
L layer					
Zn		−0·886	0·019	0·886	0·019
Pb		−0·886	0·019	0·886	0·019
Cd		−0·771	0·072	0·943	0·005
Cu		−0·771	0·072	0·943	0·005
FCP	χ^2_8	26·38		37·05	
L layer	*p*	0·001		<0·001	
F/H layer					
Zn		−0·886	0·019	0·886	0·019
Pb		−0·657	0·156	0·657	0·156
Cd		−0·886	0·019	0·886	0·019
Cu		−0·886	0·019	0·886	0·019
FCP	χ^2_8	27·50		27·50	
F/H layer	*p*	0·001		0·001	
Soil layer					
Zn		−0·886	0·019	0·886	0·019
Pb		−0·429	0·397	0·543	0·266
Cd		−0·486	0·329	0·771	0·072
Cu		−0·943	0·005	0·771	0·072
FCP	χ^2_8	22·59		21·10	
Soil	*p*	0·005		0·008	

brevicollis from the calculations for this size range leaves a predominance of spring breeders (79·2%) and analysis of this grouping of animals shows negative correlations with metal concentration (FCP: L $\chi^2_8 = 26{\cdot}38$, $p = 0{\cdot}001$; F/H $\chi^2_8 = 21{\cdot}50$, $p < 0{\cdot}001$; soil $\chi^2_8 = 22{\cdot}60$, $p = 0{\cdot}005$).

DISCUSSION

Increased litter depth in polluted woodlands has been extensively recorded, in Avonmouth (Coughtrey *et al.*, 1979) and also in other areas (e.g. in Sweden, Tyler, 1972; and North America, Strojan, 1978). The higher levels of metal concentration found in the F/H layers as compared to those in the L layers has also been recorded by Tyler (1972). The results found here are consistent with, though slightly higher than, those given by Martin *et al.* (1982) for Haw Wood.

Although no significant correlations were found between numbers of

TABLE 7
Spearman Rank Correlation Coefficients Between Metal Concentrations and Proportions of Individuals from Different Size Groups

		Size I		*Size II*		*Size III*		*Size IV*	
		Rs	*p*	*Rs*	*p*	*Rs*	*p*	*Rs*	*p*
L layer									
Zn		−0·257	0·623	0·714	0·111	−0·086	0·872	−0·812	0·050
Pb		−0·257	0·623	0·714	0·111	−0·086	0·872	−0·812	0·050
Cd		−0·143	0·787	0·543	0·266	−0·029	0·957	−0·899	0·015
Cu		−0·143	0·787	0·543	0·266	−0·029	0·957	−0·899	0·015
FCP	χ^2_8	2·85		14·09		0·72		28·78	
L layer	*p*	0·943		0·080		0·999		0·001	
F/H layer									
Zn		−0·257	0·623	0·714	0·111	−0·086	0·872	−0·812	0·050
Pb		0·086	0·872	0·657	0·156	−0·257	0·623	−0·734	0·084
Cd		−0·257	0·623	0·714	0·111	−0·086	0·872	−0·812	0·050
Cu		−0·257	0·623	0·714	0·111	−0·086	0·872	−0·812	0·050
FCP	χ^2_8	3·11		16·91		1·77		22·93	
F/H layer	*p*	0·927		0·032		0·986		0·004	
Soil layer									
Zn		−0·257	0·623	0·714	0·111	−0·086	0·872	−0·812	0·050
Pb		0·257	0·623	0·371	0·468	−0·086	0·872	−0·696	0·125
Cd		0·314	0·544	0·543	0·266	−0·486	0·329	−0·986	<0·001
Cu		−0·486	0·329	0·771	0·072	−0·029	−0·957	−0·638	0·173
FCP	χ^2_8	5·33		13·83		2·86		27·47	
Soil	*p*	0·723		0·087		0·942		0·001	

individuals or numbers of species and overall metal concentrations, negative correlations were found between species diversity (H′) and metal concentrations in the litter and soil layers. This implies that, in areas of high pollution, there is a decrease in the numbers of individuals of most species and an increase in the numbers of individuals of a few species. Lesniak (1980) reported a reduction in the proportions of rarer species and an increase in the proportions of dominant ones. In the present study, this may be due, at least in part, to the influence of *N. brevicollis*. This species was found at all six sites and in high numbers in areas near to the smelting works. In addition, a high positive correlation was found between numbers of this species and metal concentration.

Seasonal changes in the number of catches over the year also occurred. The dates of median capture were found to be later for total Carabidae, and for *A. parallelepipedus* and *N. brevicollis* in particular, in sites with higher metal concentrations. Penney (1969) reported an obligatory diapause in *N. brevicollis* which was controlled by the development of fat reserves. She also noted that this diapause could be disrupted by starvation. During the current study the diapause was seen to be absent at the most polluted site. Hengeveld (1980) recorded *N. brevicollis* as feeding mainly on mites and

Collembola and a reduction of micro-arthropods with heavy-metal pollution has been noted by Strojan (1978). In the case of other Carabidae (including *A. parallelepipedus*), low prey availability may delay peak activity. Not only is there an effect on individual species, but a reduction in the proportion of spring breeding individuals and a comparable increase in autumn breeders can also be seen. This relationship appears to be due mainly to the influence of *N. brevicollis* and may be a result of scarcity of prey. It seems unlikely that climatic factors have a major influence in delaying the activity of Carabidae. Indeed, conditions become more adverse (lower monthly temperatures and more days of frost) with increasing distance from Bristol (Findlay, 1976), and hence the pollution source.

The proportions of individual Carabidae from different size groups vary between the sites and two of the size ranges chosen were correlated with metal concentration. The increase in the proportion of size II animals in sites with higher metal contamination appears to be a direct effect of *N. brevicollis*. However, the reason for the decline of very small Carabidae is less obvious. Most of these species are spring or summer breeders and their relative decline in polluted woodlands is possibly linked to the similar decrease seen for spring breeding species overall. It is possible that an adverse effect on small arthropods, such as Collembola and mites, may also be involved here, since most of the smaller Carabidae feed on such prey.

Although gross effects on the numbers of species and the numbers of individuals of Carabidae were not to be found, differences in the ecology of this family do occur between the six sites with different contamination levels. In the present study, two major differences were observed. In those species captured from a wide range of sites, seasonal activity was later in areas of high pollution. In addition, more individual Carabidae are autumn breeders in contaminated sites. Species which are active over long periods of the year, and particularly those which have plastic activity seasons, may have an advantage in areas of high pollution. Confirmation that these observations are indeed attributable solely to the degree of metal pollution needs careful experimental examination at the species level. However, the study does illustrate the importance of examining the effect of pollution at the species level and in relation to the life histories of the animals concerned. Pollution studies that simply categorise the animals as herbivores or carnivores may miss these more subtle effects.

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APPENDIX 11.

A study of Myriapod communities in woodlands contaminated with heavy metals

by

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Summary.

The Myriapod communities of six woodlands were examined by use of pitfall traps. The results are analysed with regard to copper, zinc, lead and cadmium pollution from a nearby lead-zinc smelting works. Concentrations of metals in specimens of *Glomeris marginata* were greater at more polluted sites. Feeding experiments using juvenile *Tachypodoiulus niger* or *G. marginata* showed that metals are accumulated, affecting growth and survival rates. Experiments using adult *T. niger* indicated that the major part of the metals pass out in faeces. Results are discussed in terms of survival ability of different species at polluted sites.

Introduction.

The woodlands around Avonmouth in south west England have received much attention with regard to studies of heavy metal pollution. A more detailed description is given in Hopkin *et al.* (1985). An important difference between the polluted sites and other less contaminated woods in the area is the depth of leaf litter (Coughtrey *et al.* 1979). Hopkin *et al.* (1985) noted a large reduction in the millipede fauna in polluted woods compared to clean sites. The present study describes the myriapod community in the field and laboratory experiments using relevant millipede species.

Methods.

Myriapods were sampled in six woods ranging from 3km to 23km from the source of pollution, a lead-zinc smelter, in the direction of the prevailing winds (see Table 1). Sampling was by a grid of 15 plastic pitfall traps, containing formalin solution, at each site. Further details of sites and sampling procedures are given in Read *et al.* (1987). Traps were emptied every two weeks from 11.4.85 to 2.1.86 (19 occasions) and the captures sorted and identified. 15 samples of leaf litter were taken from each site and analysed for zinc, lead, copper and cadmium using atomic absorption spectrophotometry. Measurements of litter depth and pH were also made.

Results and discussion.

1. Analysis of communities in the field.

A total of 4283 millipedes belonging to 16 species and 565 centipedes representing 8 species were trapped (Table 1). Of immediate note is the number of millipedes from the most polluted site; 1177 individuals were caught, predominantly from three species. *Polydesmus angustus* is a widespread and common species (Blower 1985) and its presence was not unexpected. *Glomeris marginata* is listed by Hopkin et al. (1985) as absent from the contaminated Hallen wood but was caught in largest numbers at nearby Haw. The greater degree of contamination at Hallen may accounts for this difference. Chordeuma proximum was trapped only at Haw and in large numbers. This species has a predominantly southern distribution in Britain and appears to be spreading.

One noteable species absent from the most contaminated site was Tachypodoiulus niger which occurs in high numbers at some of the cleaner sites. Of the other common species of millipede found in Britain Cylindroiulus punctatus is well represented in two sites but is not expected in pitfall traps in such large numbers because its main habitat is usually dead wood. Ophyiulus pilosus was particularly abundant at only one site. Lithobius variegatus was by far the most frequent centipede and was represented in all sites.

Using Spearman rank correlation coefficients the number of individuals and species of both centipedes and millipedes were correlated with metal concentrations in the leaf litter. The only significant relationships (both positive) were between copper concentration and the number of centipedes ($p<0.05$) and cadmium concentration and the number of centipedes ($p<0.05$). Leaf litter pH had no effect on either group. However there was a significant relationship ($p<0.05$) between litter depth and number of millipedes largely explained by the numbers of G. marginata which increase with increased litter depth ($p<0.05$). When data for this species were removed, numbers of remaining millipedes were not significantly correlated with litter depth ($p>0.05$). However Hopkin et al. (1985) believed that the build up of leaf litter was caused by the absence of decomposers which were adversely affected by the metal pollution. It appears from the pitfall trapping data that this is not so, at least at the present. It is possible that in the past the number of millipede decomposers was reduced but that now the numbers are increasing due to an increase in tolerance to the pollution, or a reduction in the level of pollution, or both.

2. Metal concentrations in Glomeris marginata from different sites.

G. marginata was the only millipede species well represented at the most polluted wood and also found in all but one of the other five sites. All individuals collected in the two weeks prior to 4.7.85 were analysed using atomic absorption spectrophotometry. The majority of animals were older than stadium VI, therefore it was not possible to age them other than by weight. Results are given in Table 2. These data and those of the dry weight were examined using analysis of variance. There were significant ($p<0.01$) differences between sites for animal dry weights and for concentrations of all four metals analysed. Higher metal concentrations in the animals coincided with greater concentrations in the leaf litter samples. This suggests that the millipedes are unable to prevent the uptake of the metals into the body tissues. The findings are consistant with those of Hopkin et al. (1985) with the exception of lead. This may be due to vegetable matter in the guts of the animals which was not voided. Correcting for this material results in lead values becoming not significantly different whilst other metals are unaffected.

Pearson product moment correlation coefficients of body weights of the animals versus metal concentrations within them from the contaminated site showed significant positive correlations ($p<0.05$) with cadmium, copper and lead, ie. concentrations build up as the animals increase in age.

3. Laboratory experiments.

Both G. marginata and T. niger are readily kept in the laboratory and both species will breed sucessfully. A series of experiments were conducted to observe survival and uptake of metals in different aged individuals kept on clean and contaminated leaf litter.

Experiment 1.

Adult female T. niger were collected in June from Knapp wood which is uncontaminated and has abundant animals. The animals were kept individually in petri dishes. Each dish contained a piece of damp filter paper to maintain high humidity and three pieces of field maple leaf (Acer campestre). 6 animals were given leaves from Wetmoor (clean) and 7 were fed with Haw leaves (contaminated). All the petri dishes were placed in a plastic tank and maintained at room temperature. Dishes were observed periodically, dead animals removed, eaten leaves replaced and faeces collected. After 10 weeks all remaining animals were removed. Both the millipedes and the faeces were dried, weighed and analysed for metals. Of the 6 animals fed on clean leaves 5 were alive at the end of the experiment and of the 7 fed contaminated leaves 4 were alive. Table 3 gives mean metal concentrations for each group of animals together with mean dry weight of total faeces produced and calculated weight of faeces produced per day (ie. a correction for the animals dying early in the experiment).

None of the differences in metal concentrations between animals fed on Wetmoor leaves and those fed on Haw leaves were significant. Faeces production gives an indication of the amount each animal was eating. There was no significant difference between the total or the faeces produced per day for the different litter types. This contrasts with the results found by Hopkin et al. (1985) using adult G. marginata.

The faeces collected from each animal were analysed for metals and the results are presented in Table 4. All comparisons between food types proved significant at $p<0.05$.

When fed on polluted litter adult T. niger originating from an unpolluted site appeared able to regulate uptake of the four metals. The results show that there was no substantial decrease in feeding in the individuals fed contaminated litter, and after 10 weeks these animals did not have significantly higher levels of metals in their bodies than those fed on uncontaminated litter. Significant differences between faeces from animals fed on contaminated and uncontaminated litter occurred for all four metals. These results are in contrast to adult G. marginata (see section 2 above).

Experiment 2.

Adult T. niger from Knapp wood collected in 1986 were maintained as pairs on their own leaf litter. At the end of May, two females laid nests of eggs which subsequently hatched. Offspring from these nests, numbering 84 in all, were removed when they reached stadium II and placed in petri dishes, 6 individuals to each dish and assigned to one of two groups. The experiment ran for 14 weeks, any individual dying was removed, aged and dried for analysis. Animals alive at the termination of the experiment were treated likewise.

Group 1 - Petri dishes contained damp filter paper and field maple leaves from Wetmoor (clean).

Group 2 - Filter paper and field maple leaves from Haw (contaminated).

Both families were treated in this way, but as the results between the families were not significantly different they are combined in the following analysis. It was obvious from the begining that the food intake of the animals in group 1 was far greater than those in group 2. Figure 1 shows the survivorship curves for animals fed on the different leaves whilst Figure 2 gives the stadial age at death for each group. It is clear that the mortality rate and the stadial age differ greatly according to the food type. A Mann-Whitney U test was calculated to compare the median date of death for each group and the median age at death. Both were significantly different ($p<0.001$) thus animals fed on contaminated litter die earlier and grow more slowly than those fed on clean litter.

The dry weights and metal concentrations of each group are shown in Table 5a. In all cases the animals fed on the contaminated leaves had significantly higher concentrations of metals in their tissues than those fed on clean leaves, implying that they are unable to regulate the quantity unlike the adults. Similar results were obtained by Hopkin & Martin (1984) with the woodlouse _Oniscus asellus_. In _T. niger_ there were differences also in dry weight, indicating that growth was much slower in animals fed on contaminated litter.

Experiment 3.

Experiment 2 was repeated using juvenile _G. marginata_ bred from a single female collected at Knapp wood. Stadial age at death proved too difficult to establish. Figure 3 shows the survival of the two groups. The test of the medians was significant at $p<0.01$. The results of the metal analysis are shown in Table 5b. Again there were significant differences in dry weight showing the slower growth rate of individuals fed on contaminated litter. Significant differences occurred between groups for concentrations of cadmium and zinc but not copper and lead.

Conclusions.

The community structure of woodland millipedes in the sites investigated appears to be very variable and many factors are obviously involved in determining which species occur where. One way to assess the effects of pollution is to examine the species that are present or absent. An effect of increasing dominance of a few species seems to occur, as may be expected if a small number of species are able to become tolerant to the pollution. _G. marginata_, recorded in large numbers in one polluted site but apparently absent from another (Hopkin _et al_. 1985) was found to accumulate metals in relation to age of individual and concentration in the leaf litter. Young animals from clean sites quickly accumulated cadmium and zinc when fed on contaminated litter and grew significantly more slowly than those fed on clean litter.

T. niger which is absent from the polluted sites could be fed sucessfully as adults on contaminated litter without accumulating metals. However juveniles had a low rate of survival, a slow growth rate and a fast accumulation of metals when fed contaminated litter.

Survival of these millipedes in polluted sites appears to be most acute in the young stages. _G. marginata_ seems to have overcome this problem and was found in some of the polluted woodlands whereas _T. niger_ which can survive as adults but not as juveniles has not. Further studies of _G. marginata_ from contaminated sites may indicate how this species survives. It is peculiar that _T. niger_ with its vagile habits and interesting life cycle (Blower & Fairhurst 1968) is the species which has not been able to colonise these polluted areas. The reason cannot lie in the difference in generation time, ie. a fast turnover giving a faster rate of evolution of tolerance, as _G. marginata_ takes 3 to 4 years to reach maturity (Heath _et. al_. 1974) whilst for _T. niger_ it is usually 3 years (Blower & Fairhurst 1968). It may be only a matter of time before _T. niger_ occurs in the polluted areas.

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Table 1.

Numbers of millipedes and centipedes caught at each site together with details of the sites.

Species/details	SITES W.M.	K.W.	K.G.	T.P.	P.W.	H.W.*
Distance from smelter (km)	23	19	14	12	8	3
Cd in litter (ug/g)	0.6	1.0	2.0	1.7	3.3	19.9
Cu in litter (ug/g)	12.2	13.7	18.2	17.6	23.9	151
Pb in litter (ug/g)	32.8	50.0	98.0	125.6	137.9	1142.4
Zn in litter (ug/g)	74.4	112.8	159.2	193.8	257.0	1389.8
Litter depth (mm)	32	61	86	44	85	131
pH	5.2	5.4	4.4	5.1	4.9	4.8
Millipedes.						
Glomeris marginata (Villers)	53	95	270		308	482
Nanogona polydesmoides (Leach)			27	66	3	16
Chordeuma proximum Ribaut						313
Isobates varicorne C.L. Koch	5	2		1		
Proteroiulus fuscus (Am Stein)	1		43	37		
Ommatoiulus sabulosus (Linne)			1			
Tachypodoiulus niger (Leach)	51	452	159	89	9	
Cylindroiulus punctatus (Leach)	3		63	93	8	
C. britannicus (Verhoeff)			12			
Julus scandinavius Latzel			112		44	
Ophyiulus pilosus (Newport)		197	6			
Brachyiulus pusilus (Leach)				1		
Polydesmus angustus Latzel	1	1	278			216
P. gallicus Latzel	36	111	26	79		
P. denticulatus C.L.Koch	1	95				75
Brachydesmus superus Latzel	3	61	23	5	2	20
Imm. Julidae	10	25	25	2	28	
Imm. Polydesmidae	1	5	66	8	5	55
No. of species	9	8	12	8	6	6
No. of individuals	164	1044	1111	380	407	1177
Centipedes.						
Strigamia crassipes (C.L.Koch)	11					
Strigamia accuminata (Leach)					4	2
Brachygeophilus truncorum (Bergso & Meinert)		2	3	5		
Lithobius variegatus Leach	69	41	75	53	116	93
Lithobius forficatus (Linn.)	11	3		12	5	2
Lithobius borealis Meinert	1	-	1	2		
Lithobius crassipes L.Koch	2		1		1	12
Lithobius microps Meinert	1		3	3	3	4
Imm. Lithobius sp.	1	1	1	1	16	1
No. of species	6	3	5	5	5	5
No. of individuals	96	47	84	76	145	117

W.M. = Wetmoor wood K.W. = Knapp wood K.G. = Kington Grove
T.P. = Tockington Park P.W. = Pegwell wood H.W. = Haw wood

Table 2.

Metal concentrations (ug g^{-1} +S.E.) in _G. marginata_ (dry weight in mg) from five sites.

Site	n	Dry weight	Cadmium	Copper	Lead	Zinc
W.M.	23	21.49+3.34	15.44+1.68	45.81+3.53	4.81+1.3	579.3+29.1
K.W.	27	9.25+0.94	3.86+0.56	32.86+1.74	11.44+5.43	534.9+30.3
K.G.	27	28.14+4.27	4.42+0.38	47.60+14.8	12.43+4.98	488.4+19.1
P.W.	28	12.24+1.61	7.41+0.88	51.09+2.36	32.42+5.19	564.3+19.0
H.W.	66	17.98+1.9	26.49+0.74	71.53+1.1	30.09+2.91	714.8+10.9

One way analysis of variance between sites for all variables were significant at p<0.01.

Table 3.

Metal concentrations (ug g^{-1}) and faeces weights in adult _T. niger_ fed on different litter types.

	Wetmoor fed	Haw fed	Significance of t
n	6	7	
Cadmium	1.06+0.36	3.09+1.36	NS
Copper	179.70+12.4	217.20+26.2	NS
Lead	0.51+0.17	3.28+1.19	NS
Zinc	336.60+96.8	458.0+74.40	NS
Mean faeces weight (mg)	40.60+14.1	10.28+3.30	NS
Mean faeces/ day (mg)	0.83+0.219	0.28+0.14	NS

NS = not significant at p = 0.05.

Table 4.

Metal concentrations in faeces from adult _T. niger_. (ug g^{-1})

	Wetmoor fed	Haw fed	Significance of t
n	27	28	
Cadmium	1.57+0.20	94.0+2.41	**
Copper	70.00+11.2	121.3+21.1	*
Lead	23.47+3.84	388.0+107	**
Zinc	226.40+30.7	721.0+135	*

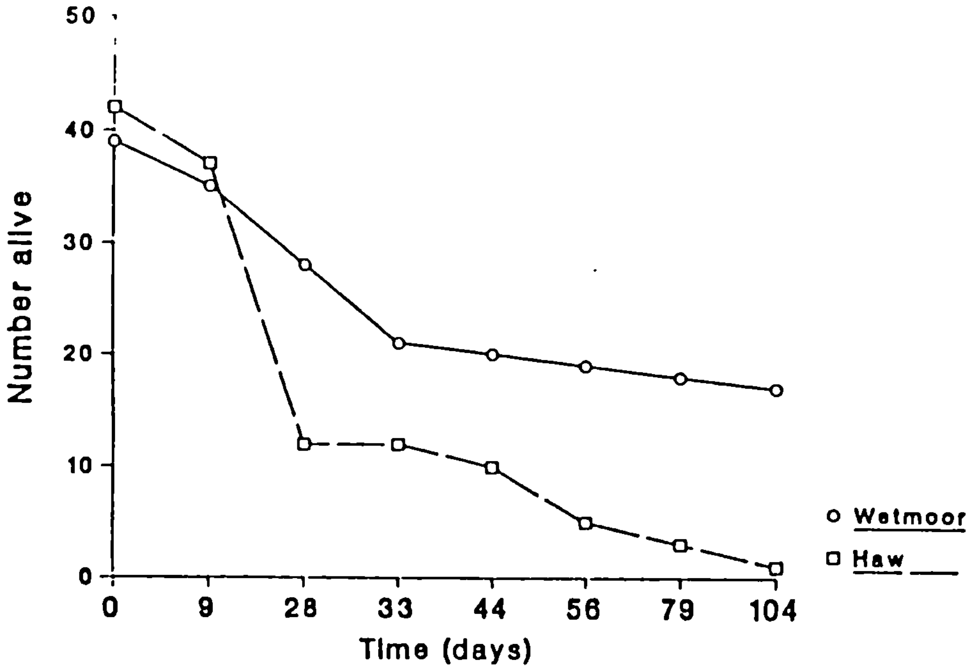
* = p<0.05 ** = p<0.01

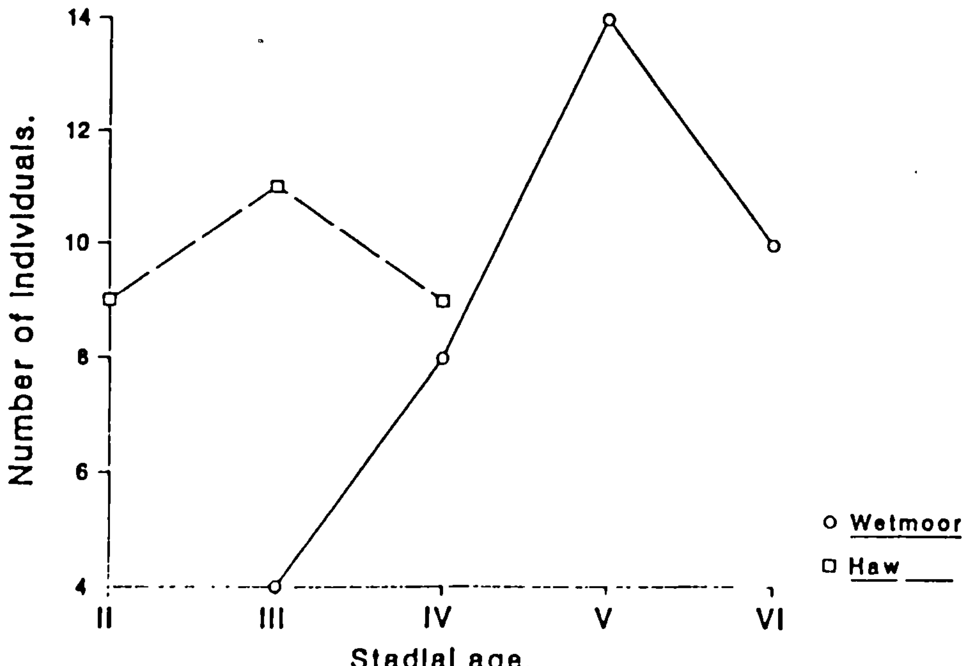
Table 5.

Weights (mg) and metal concentrations (ug g^{-1}) in juvenile *T. niger* and *G. marginata* reared on different litter types.

	Wetmoor fed (group 1)	Haw fed (group 2)	Significance of t
a. *T. niger*			
n	36	34	
Weight (mg)	0.86+0.107	0.18+0.022	***
Cadmium	0.75+0.11	8.72+1.45	***
Copper	200.0+12.3	569.5+40.2	***
Lead	22.2+7.07	228.8+36.3	***
Zinc	607.6+38.5	1363.7+96.2	***
b. *G. marginata*			
n	36	39	
Weight (mg)	0.36+0.059	0.21+0.013	*
Cadmium	8.83+1.06	11.54+0.84	*
Copper	75.90+13.0	97.70+15.9	NS
Lead	62.00+14.6	185.10+73.6	NS
Zinc	827.20+68.9	1052.30+59.4	*

NS = not significant at p = 0.05 * = p<0.05 *** p<0.001


50
40
30
20
10
0
Number alive
0
9
28
33
44
56
79
104
Time (days)
o Wetmoor
□ Haw ___


14
12
10
8
6
4
Number of Individuals.
II
III
IV
V
VI
Stadial age.
o Wetmoor
□ Haw ___

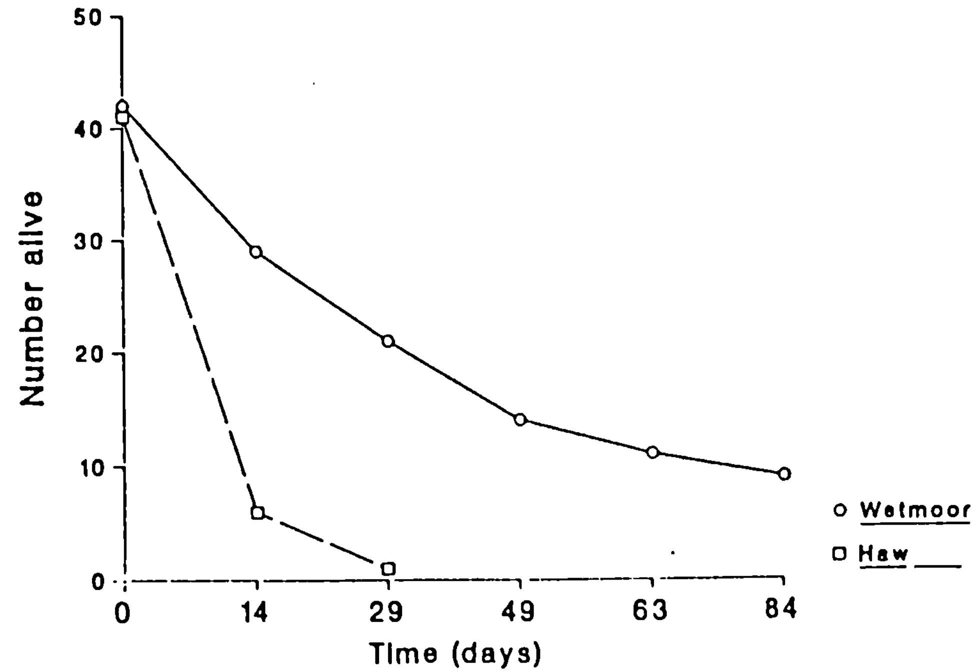

50
40
30
20
10
0
Number alive
0
14
29
49
63
84
Time (days)
o Wetmoor
□ Haw ___

Figure 1.
Survivorship curves for juvenile _Tachypodoiulus niger_ fed on _Acer campestre_ leaves from Wetmoor (clean) or Haw (contaminated).

Figure 2.
Stadial age at death (or termination) for juvenile _T. niger_ fed on leaves from Wetmoor and Haw.

Figure 3.
Survivorship curves for juvenile _Glomeris marginata_ fed on leaves from Wetmoor (clean) or Haw (contaminated).

APPENDIX 12.

The life history of *Chordeuma proximum* Ribaut. from a wood in Avon.

by

Helen J. Read.

Introduction and methods.

Four species of the family Chordeumatidae are found in Britain and Blower (1984) has detailed the distinctive features of each of them. In the later synopsis Blower (1985) notes that *Chordeuma proximum* appears to be an annual species in the south, whereas in the Forest of Dean there is a suggestion of a two year life cycle. Whilst undertaking a long term pitfall trapping study in various sites in Avon, one woodland was discovered to contain a population of *C. proximum*. As trapping continued for a year this was a good opportunity to look in more detail at this species.

Haw wood (ST 558 800) where the collections were made is a mixed deciduous wood containing oak, hazel, field maple and large quantities of brambles. The leaf litter is exceptionally deep, averaging 131 mm in depth. The wood is 3 km down wind of a large zinc smelting works at Avonmouth and consequently the leaf litter and the mineral soil have greatly elevated levels of zinc, cadmium, lead and copper.

15 pitfall traps (plastic vending machine cups) were laid in a 5 x 3 grid formation. Each contained 5 ml of a 4% formalin solution containing a few drops of detergent to reduce surface tension. They were emptied at fortnightly intervals (weather permitting). Captures from each trap have been lumped into trapping occassions for this study. Collecting by pitfall trapping has the disadvantage that animal must be mobile to have a chance of being caught. Captures are therefore the result of abundance and activity. Consequently the

younger stadia which, being less active, are less likely to be caught and will be under represented in the data. However, from those individuals that were captured, the stadia from v to ix can be characterised and some comments on life histories made.

Ocular field in C. proximum.

As noted in Blower (1984) the ocular field in Chordeuma spp. is built up by adding an additional line of ocelli with each moult. Commencing with two rows containing only one ocellus, a subsequent row of two is added at the moult to stadium iv, a row of three to stadium v and so on. Ideally an equilateral triangle is built up with one extra ocelli at one point (Figure 1). In some instances the eyes will become reduced, that is, the full compliment of ocelli will not appear. Blower (1984) suggests that C. proximum is more likely to have incomplete rows than Chordeuma sylvestre and illustrates the growth of the ocular field of Melagona scutellare (Chordeumatidae) in which there is a considerable reduction. Figure 2 illustrates the ocular field from stadium v in C. proximum from Haw. The majority of specimens showed the maximum compliment of ocelli up to stadium viii but a reduction of one in the final row in stadium ix. This final row often consisted of smaller ocelli and the ventral most ocellus was difficult to see and squashed in between the ventral margin, the Organ of Tomosvary and the other ocelli. Thus in specimens from Haw wood there does not appear to be a great reduction in the numbers of ocelli.

Life cycle.

The animals captured were assigned the correct stadium by using the ocular field and the numbers in each stadia are recorded in Table 1 for each trapping occassion.

From 4.7.85 the growth of the 1985 cohort of animals can be followed up to maturity in October. In this period there are no adult specimens caught. At all other times of the year there are adults, including mature males. This is indicative of a single year life cycle.

In order to determine oviposition time, females from various collections were dissected for eggs. A maximum of 54 eggs were found between segments 10 and 28 (30 being the telson) in a female caught on 23.5.85. Whilst large eggs were present in females during the spring, none were found in females caught in October. Very small eggs were noted from animals from November. This confirms the suggestion from Table 1 that oviposition occurs in the late spring.

It would appear that the population of C. proximum in Haw wood is annual and there is no indication of a two year life cycle as in the Forest of Dean animals (Blower 1986). In the year of this study (1985) the population was also well synchronised, in contrast to C. sylvestre in Cornwall. This species in April yielded adults and newly hatched stadia ii, an observation which was presumed to be due to an extended period of egg laying (Blower 1986). At Haw the period of egg laying seems to be more precise.

References

Blower, J. G. (1984). The British Chordeumatidae. Bull. Br. Myriap. Gp. 2:8-23.

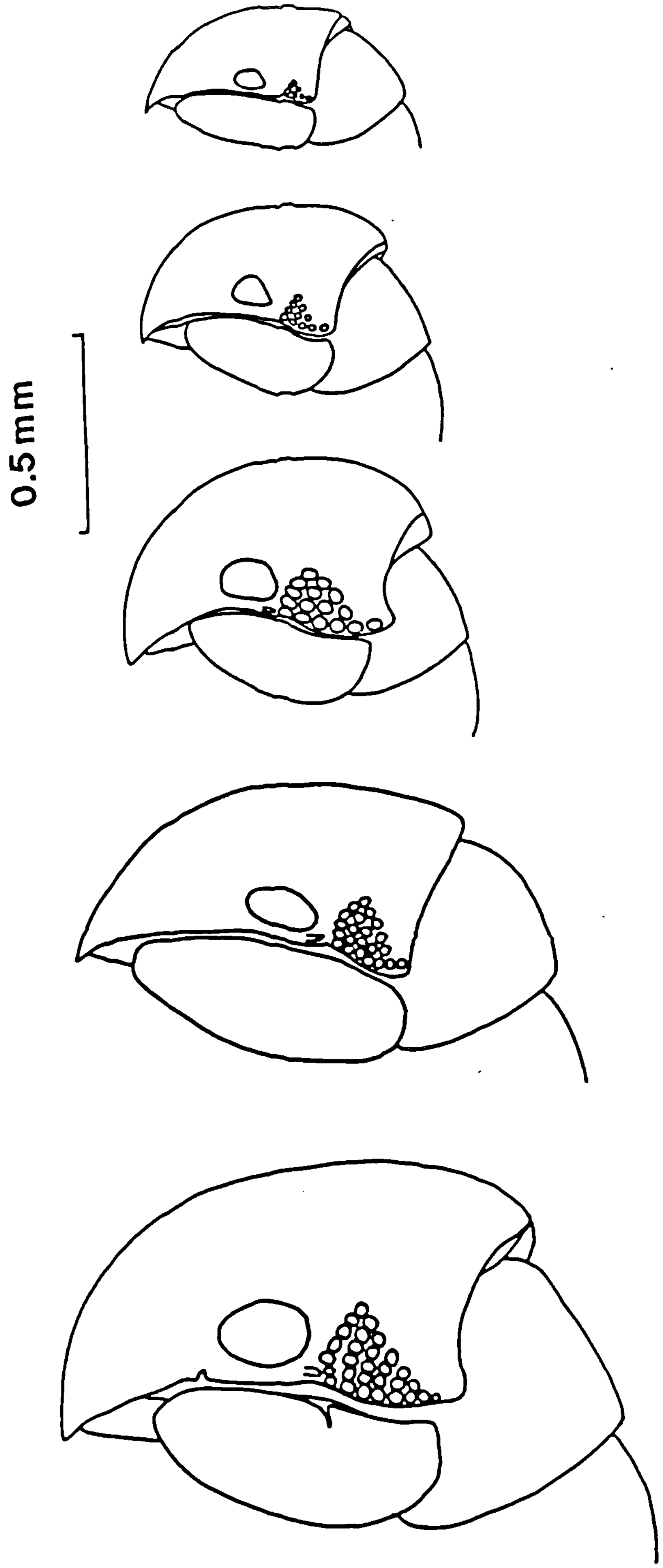
Blower, J. G. (1985). Millipedes. Synop. Br. Fauna (New Series) No. 35. E. J. Brill/Dr. W. Blackhuys publishers.

Table 1.

Numbers of Chordeuma proximum caught at Haw.

			STADIUM		
Date	v	vi	vii	viii	ix
19.12.84				1	
02.01.85				3	17
23.01.85				1	13
31.01.85				1	19
28.02.85				3	31
14.03.85				1	18
28.03.85					4
11.04.85					1
25.04.85					3
09.05.85					17
23.05.85					8
06.06.85					8
20.06.85					
04.07.85	2				
18.07.85		6			
01.08.85			6		
15.08.85			11		
29.08.85			7	1	
12.09.85			2	35	
26.09.85			1	8	
10.10.85				1	11
24.10.85				3	40
07.11.85				4	42
21.11.85				7	13
05.12.85				5	3
19.12.85				2	16

Dates given are those when the traps were collected; they were in position for the entire duration between collecting times, usually a period of two weeks (including the collection on 19.12.84.)



V

VI

VII

VIII

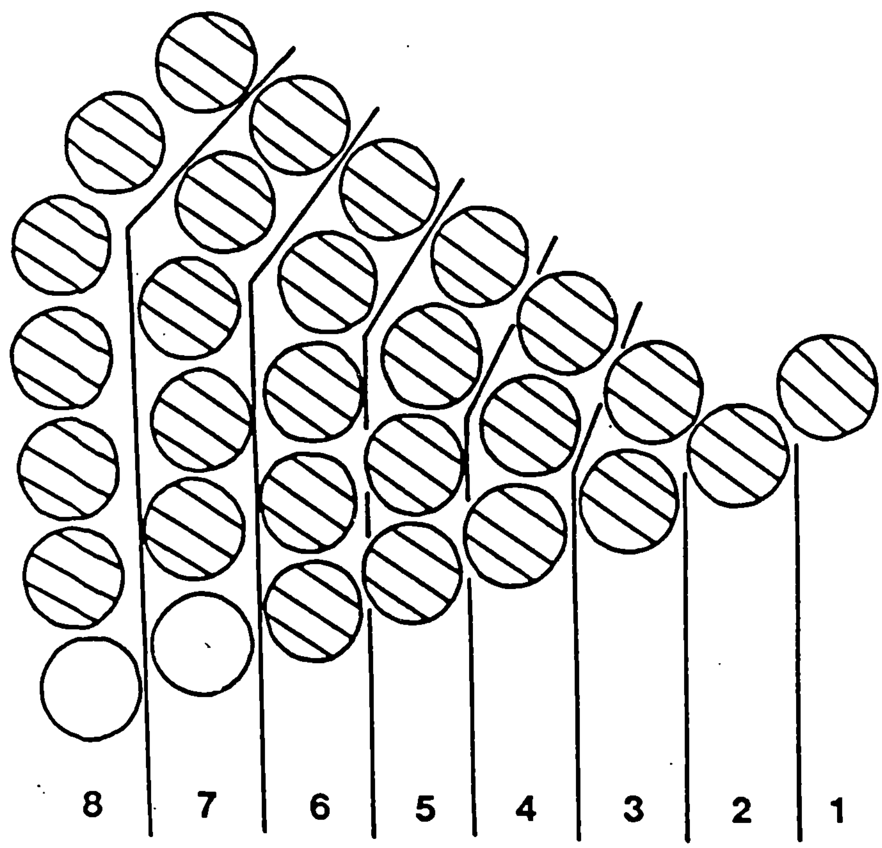
IX

Figure 1.

Diagramatic representation of the ocular field. Ocelli cross hatched are those always found in animals from Haw, those left open are present in some animals but not all. Numbers indicate the order in which the rows are added.

Figure 2.

The ocular fields in C. proximum from stadium v to stadium ix.


8
7
6
5
4
3
2
1